

# POTENTIAL OF CHITOSAN-BASED HYDROGELS WITH HORMONES AND GROWTH FACTORS IN CHRONIC WOUND THERAPY – THE POSSIBILITIES OF PREPARING A PRESCRIPTION DRUG BASED ON A LITERATURE REVIEW

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## Abstract

Chronic wounds are characterised by delayed healing and an atypical healing process that stops at the inflammatory or proliferative phase. The aim of this article is to indicate the potential of chitosan hydrogels encompassing hormones and growth factors in the treatment of chronic wounds and indicate their possibility as a prescription drug. A comprehensive search was conducted across Google Scholar, PubMed, Scopus, and Web of Science databases, identifying eight studies on chitosan hydrogel preparation technology as potentially suitable for the manufacture of a pharmacy formulation setting. The literature review indicates that chitosan hydrogel carriers containing hormones or hormone derivatives may be used in the treatment of hard-to-heal wounds. They are exceptional primarily due to their favourable physicochemical properties, allowing epidermal application, simplicity of preparation, and low manufacturing cost. However, the lack of clinical trials suggests the need for further studies on their efficacy and safety of use.

**Keywords:** chitosan, hormone, growth factors, hydrogel, dermal drug delivery, wound dressing

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## **1. Introduction**

Personalised medicine allows treatment to be tailored to the patient, with their individual needs in mind. The foundation of personalised medicine is prescription medicines. Preparing prescription medicines for difficult-to-heal wounds allows for tailoring the treatment to the individual needs of the patient, taking into account their age, gender, wound aetiology, microbiological condition of the wound, stage of the disease, presence of co-morbidities, medications taken, and occurrence and intensity of pain. This makes it possible to precisely select the drug dosage, develop a formulation with a unique composition and limit the number of excipients, including the elimination of potential allergens. Thus, it is possible to tailor the composition and properties of the formulation to the different stages of wound healing. Particularly for infected wounds, it is important that the preparation has an antimicrobial effect, absorbs the odour emitted by the wound, protects against the development of infection in the wound, and absorbs excess moisture [1]. In recent years, there has been a significant increase in research on chitosan hydrogels for accelerating wound healing, indicating the potential of using them as a carrier in prescription medicines.

Chitosan is a natural biopolymer produced on an industrial scale from shells of crustaceans. It exhibits biocompatibility, biodegradability, and antimicrobial activity, making it a highly promising material for the treatment of dermatological diseases. It primarily promotes cell proliferation and angiogenesis. The role of chitosan in accelerating wound healing was described in detail in our recent article [2]. The incorporation of hormones and growth factors in chitosan hydrogels offers new opportunities in the treatment of chronic wounds by influencing healing processes at the cellular level. Most of the current research focuses on developing a carrier with appropriate quality parameters to ensure stability and optimal release and penetration of API active substances.

This review fills a gap in the literature by providing pharmacists with practical information on the potential use of chitosan in the pharmacy setting. It demonstrates the potential of this raw material for the preparation of dermatological hydrogel preparations in the pharmacy, taking into account the actual technological capabilities of pharmacies and current data on the safety, efficacy and stability of chitosan-containing preparations.

The aim of this article is to provide insight into the potential of chitosan hydrogels with hormones and growth factors in the treatment of chronic wounds, with a view to developing prescription drugs.

## **2. Materials and Methods**

Four authors searched Google Scholar, PubMed, Scopus, and Web of Science databases independently. Articles were searched for studies on the therapeutic efficacy of epidermal chitosan preparations with hormones and growth factors in the treatment of chronic wounds. Keywords included: chitosan, biopolymers, polysaccharides, hormone, growth hormone, hydrogel, hydrogel dressing, wound dressing, bioactive dressings, skin regeneration, topical therapy, dermal drug delivery, transdermal penetration, burn, wound healing, promoting cell growth, diabetic foot ulcers, burn wound healing. Inclusion criteria were adopted: original English-language articles published in peer-reviewed journals between 2000 and 2025. Studies on chitosan hydrogels in terms of their potential use in the production of prescription drugs were also taken into account. Exclusion criteria: articles in which the chitosan-insulin preparation was administered by a route other than

epicutaneous and articles describing the technology of hydrogel preparation that could not be performed in a pharmacy setting.

### 3. Review of Research on the Development of Chitosan Formulations with Hormones and Growth Factors to Promote the Healing of Chronic Wounds

Chronic wounds are skin lesions whose healing process is impaired, i.e., the healing process is abnormal over a significantly prolonged period of time. It is usually assumed that the inflammatory phase persists for more than six weeks and that the wound recurs despite the treatment [3]. Chronic wounds are a consequence of complications of various diseases and abnormalities of the circulatory system. The problem increases particularly for people who suffer from hyperglycaemia and neurological diseases. The elderly are also at increased risk [4]. Chronic wound management is a complex problem involving health, economic, and epidemiological sectors [3, 4]. It is estimated that 64% of people receiving wound care at home have wounds of chronic aetiology in Europe [5].

Table 1 summarises the studies (basic and preclinical) included in this review covering the therapeutic efficacy of dermatological preparations containing hormones or growth factors in a chitosan-based hydrogel carrier for the treatment of chronic wounds. Studies were selected in which the hydrogel preparation technology demonstrated the potential for its manufacturing in a pharmacy formulation setting.

#### 3.1. Chitosan Hydrogels with Insulin

Insulin (INS) exhibits antioxidant and anti-inflammatory effects. It can promote keratinocyte migration and proliferation via the IR/PI3K/Akt pathway, increasing, among others, the expression of the  $\alpha 3$  subunit of integrin, which promotes wound healing. Through the IGF-1 receptor, insulin induces the formation of collagen, a key component of the extracellular matrix (ECM). It also exhibits pro-angiogenic effects by regulating the migration, proliferation, and formation of endothelial cells, increasing the expression of the vascular endothelial growth factor VEGF and decreasing the expression of anti-angiogenic proteins. In addition, insulin has anti-inflammatory effects, modulating NF- $\kappa$ B (nuclear factor kappa B transcription factor) and the expression of pro-inflammatory cytokines. It also induces the phagocytic activity of neutrophils, with an antimicrobial effect [2, 6–9].

Ostróžka-Ciešlik *et al.* [6] designed an insulin-containing hydrogel (INS) based on chitosan (CS; medium molecular weight, deacetylation degree of 75 - 85%; viscosity of 200 - 800 cP) in a system with cellulose derivatives (methylcellulose, MC; hydroxyethylcellulose, HEC; hydroxypropylmethylcellulose, HPMC). The research focused on the development of a formulations of the dermatological insulin drug. The formulations developed showed a prolonged hormone release profile. The amount of INS released decreased in a series: CS/HPMC+INS (49%) > CS/HEC+INS (42.5%) > CS/MC+INS (39.8%). INS release followed the Peppas-Sahlin model (transport by diffusion and erosion). The viscosity of the preparations decreased in order: CS/MC+INS ( $\eta_{30} \text{ s}^{-1} = 14.0 \text{ Pa}\cdot\text{s}$ ) > CS/HEC+INS ( $\eta_{30} \text{ s}^{-1} = 5.81 \text{ Pa}\cdot\text{s}$ ) > CS/HPMC+INS ( $\eta_{30} \text{ s}^{-1} = 4.23 \text{ Pa}\cdot\text{s}$ ). The rheological characteristics of the formulation were close to the Herschel-Bulkley model ( $R^2 = 0.997 - 0.999$ ). In oscillatory tests done at 25°C and 32°C, a trend of  $G' < G''$  was observed, indicating a fluid consistency ('viscoelastic liquids') of the formulations. This suggests that hydrogels can adapt to the shape of the wound without compromising their properties. The low values of the 'hardness 1' parameter in the texture

**Table 1.** Review of studies on chitosan preparations with hormones and growth factors.

Hormone/growth factor (dose)	Hydrogel substrate	Research model	Results	[Ref] Year
insulin (28.57 IU/g)	chitosan (2% w/w) - methylcellulose (4% w/w); chitosan (4% w/w) - hydroxyethylcellulose (2% w/w); chitosan (2% w/w) - hydroxypropyl methylcellulose (4% w/w)	<i>in vitro</i> pharmaceutical availability studies using Strat-M® membrane; rheological analysis; texture analysis	hormone release from the formulation occurs in a prolonged manner; hydrogels are shear-thinning non- Newtonian fluids with a flow limit; hydrogels have a fluid consistency (viscoelastic liquids) at 25°C and 32°C ( $G' < G''$ ); an acceptable balance between rheological and texture parameters and ease of application was found	[6] 2024
insulin (2 U/g)	chitosan - hydroxypropyl methylcellulose (2:1 v/v)	FTIR, TGA, and SEM studies; <i>in vitro</i> - assessment of cell viability in a human keratinocyte (HaCat) cell model using MTT and wound scratch tests; <i>in vivo</i> - wound model in mice with induced diabetes	CS/HPMC+INS hydrogel induced no cytotoxicity; the formulation promoted: faster wound closure in the scratch test, granulation tissue formation, hair follicle regeneration, increased keratinocyte migration	[10] 2024

*Abbreviations:* DSC, differential scanning calorimetry; EGF, epidermal growth factor; FGF, fibroblast growth factors; FTIR, fourier transform infrared spectrometry; G, glycerol as a softener; GH, somatotropin or growth hormone; GN; chemically cross-linked by Genipin; HA, hyaluronic acid; MTT, 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; PEGDA, poly(ethylene glycol) diacrylate; PVA, polyvinyl alcohol; SA, alginate; SEM, electron microscopy; TGA, thermogravimetric analysis; VE-cadherin, vascular endothelial cadherin; ZnG, zinc gluconate.

**Table 1.** (continued) Review of studies on chitosan preparations with hormones and growth factors.

Hormone/growth factor (dose)	Hydrogel substrate	Research model	Results	[Ref] Year
somatotropin (25 mIU)	chitosan - polyvinyl alcohol - glycerol (optimum formulation of CS-PVA-G is 68:30:2 w/w)	<i>in vitro</i> : microscopy, SEM, DSC, FTIR, degradation tests, MTT test, release test	the hydrogel ensures a moist wound site with optimum mechanical strength; prolonged release of GH; somatotropin in the hydrogel increases the percentage of cell survival	[11] 2024
EGF (10 µg/ml)	chitosan (2%)	<i>in vitro</i> : pharmaceutical availability study using a dialysis bag; <i>in vivo</i> : Sprague-Dawley rat burn wound model, histological, histochemical, and immunohistochemical studies	prolonged release of EGF from the hydrogel (Q = 97.3% after 24 hours); increased cell proliferation; improved and faster epithelialisation	[12] 2006
rhEGF (20 µg/ml) ZnG (0.5% w/v)	chitosan (2% w/v) poloxamer 407 (23% w/v)	<i>in vitro</i> : microscopic analysis, rheological analysis, <i>in vitro</i> release, antibacterial activity, antibiofilm activity; <i>in vivo</i> : Sprague-Dawley rat burn wound model, histological and biochemical studies	developed hydrogel is cytocompatible and non-irritant; demonstrated inhibitory activity against <i>S. aureus</i> and <i>P. aeruginosa</i> and against their biofilms; promotes vascular remodeling and collagen deposition; facilitates fibrogenesis and reduces IL-6 levels	[13] 2021

**Table 1.** (continued) Review of studies on chitosan preparations with hormones and growth factors.

Hormone/growth factor (dose)	Hydrogel substrate	Research model	Results	[Ref] Year
bFGF (100 ng/mL), VE-cadherin (20µg/mL)	chitosan (20 mg/mL) - alginate (20 mg/mL) - poly(ethylene glycol) diacrylate (100 mg/mL)	<i>in vitro</i> : swelling factor analysis, degradation factor; rheological test; assessment of haemolysis and dynamic coagulation in whole blood; <i>in vitro</i> epithelial cell compatibility assessment; <i>in vitro</i> and <i>in vivo</i> antimicrobial activity; <i>in vivo</i> : Sprague-Dawley rat wound model	developed hydrogel induced cell adhesion, proliferation, and migration; had optimal haemostatic and antimicrobial properties; promoted wound healing in a full-thickness skin defect model	[14] 2022
bFGF (100 ng/mL), VE-cadherin (20µg/mL)	chitosan (20 mg/mL) - alginate (20 mg/mL) - poly(ethylene glycol) diacrylate, CS - PEGDA mixed at a ratio of 2:1 v/v	<i>in vitro</i> : FTIR studies, rheological analysis, stability studies; <i>in vivo</i> : burn wound model (shallow and deep) in the rat	bFGF and VE-cadherin showed synergistic effects developed hydrogel promoted cell proliferation and adhesion; enhanced cell-cell connections and communication; increased cell viability; promoted faster wound healing	[15] 2025
FGF (2.64 · 10 <sup>-6</sup> M)	chitosan (2% w/v) - hyaluronic acid (1% w/v) – AuNPs (Au, nanoparticles)	<i>in vitro</i> : swelling test, biodegradation, antimicrobial activity, cell viability, cell migration	increased cell survival (> 90%) cell proliferation and migration capacity high antibacterial efficacy against Gram-positive and Gram-negative pathogens	[16] 2024

test (0.048 - 0.081 N,  $p < 0.05$ ) indicates that hydrogels are easily spread on the skin. It is noteworthy that the authors introduced a ready-made human insulin preparation (Insulin Insulatard Penfil) into the chitosan formulation, containing, among others, metacresol and phenol with antimicrobial activity, which made the hydrogel self-protecting from microbial contamination. The authors suggested that the proposed hybrid hydrogel matrices could be an effective carrier of insulin and promote the healing process of chronic wounds.

Zanchetta *et al.* [10] prepared an insulin-containing hydrogel based on chitosan (molecular weight of 190 - 310 kDa, deacetylation degree of  $\geq 75\%$ ) and HPMC (2:1 v/v). In the MTT test, it was found that the CS/HPMC/INS hydrogel showed no cytotoxicity to human keratinocytes and, importantly, increased mitochondrial viability (the test was conducted after 24, 48, and 72 h of contact). In the wound scratch test (0 - 60 h), on the other hand, the complete wound closure was confirmed 60 hours after application ( $p < 0.001$ ). The hydrogel stimulated cell proliferation and migration of HaCaT (immortalised human keratinocytes). The group treated with CS/HPMC/INS hydrogel showed more organised granulation tissue compared to the control groups in histological evaluation. On the twentieth day after the injury, complete re-epithelialisation, a smaller scar area, and hair follicle growth were confirmed. Importantly, epidermal administration of insulin through the CS/HPMC vehicle did not affect blood glucose levels.

### 3.2. Chitosan Hydrogels with Somatotropin (Human Growth Hormone, hGH)

Somatotropin stimulates the proliferation and differentiation of skin cells. It participates in protein synthesis, collagen production and angiogenesis, thereby promoting healing processes [17]. It is suggested that GH stimulates the production of IGF-1, which induces fibroblast proliferation and keratinocyte migration at the wound site [18]. Clinical studies confirm its effectiveness in burn wound healing, for instance, in paediatric patients [19]. Nevertheless, there are suggestions that patients treated with growth hormone in childhood may have an increased risk of developing cancer [20].

Nazerian *et al.* [11] developed a hydrogel carrier for somatotropin. It was based on chitosan (CS, 75 - 85% deacetylation degree and an average molecular weight of 190 - 310 kDa) and polyvinyl alcohol (PVA, hydrolysis degree of 98% and an average molecular weight of 72,000 g/mol), which were chemically cross-linked by Genipin (GN, 0.5 wt.%), and contained glycerol as a plasticiser. The hydrogel showed optimal physicochemical parameters. After a time of 1 h and 45 min, 0.014  $\mu\text{g/mL}$  of somatotropin was released from the carrier. The release process occurred according to first-order kinetics. The hormone incorporated into the hydrogel matrix caused an increase in the cells' survival. In addition, somatotropin caused no toxicity and contributed to the growth of fibroblast cells.

### 3.3. Chitosan Hydrogels with Epidermal Growth Factor (EGF)

Epidermal growth factor (EGF) participates in cell regeneration processes by stimulating the proliferation of epidermal cells (that form the epidermis) and cells lining the endothelium, as well as influencing the activity of fibroblasts. EGF's mechanism of action is related to the stimulation of its receptor of EGF tyrosine kinase, which further activates a cascade of cell signalling for proliferation of, among others, keratinocytes involved in the restoration of the epithelial layer [21, 22]. There are suggestions that keratinocytes in chronic wounds have reduced levels of EGFR, which hinders their response to this receptor signalling and, in turn, impairs the healing process, contributing to the persistence

of chronic wounds. EGF at low concentrations (1 ng/ml) has been found to stimulate cell migration, whereas at high concentrations (i.e., 100 ng/ml) it has an inhibitory effect [23].

Alemdaroğlu *et al.* [12] used 2% chitosan matrices as a carrier for an epidermal growth factor (EGF). The authors analysed the effectiveness of the developed formulation in promoting the healing of second-degree burn wounds in a rat model. In an *in vitro* pharmaceutical availability study, they found that EGF was released from the hydrogel in a prolonged manner (97.3% at 24 h) according to first-order kinetics. In an *in vivo* study, rats with an induced burn wound were treated for 14 days with the EGF-containing hydrogel (0.16 µg/cm<sup>2</sup> dose, replaced daily). An increase in cell proliferation ( $p < 0.001$ ) and epithelialisation rate ( $p < 0.001$ ) was observed. The presented data suggest that the EGF-containing hydrogel is effective in wound healing.

Lin *et al.* [13] developed a thermosensitive hydrogel based on chitosan (100 kDa; degree of deacetylation > 95%; viscosity of 109 mPa.s) and Poloxamer 407 with incorporated zinc (zinc gluconate) and recombinant human epidermal growth factor rhEGF. The developed formulation (ZnG/rhEGF/CS/Pol 407) underwent reversible transformations with respect to temperature change – crosslinking at 37°C and dissolution at 4°C. During the phase transition at  $T = 37^{\circ}\text{C}$  the viscosity increased and the total gelation time was 0.42 min. The formulation was characterised by the optimal mechanical strength. API release occurred in a controlled manner, according to zero-order kinetics. Moreover, the ZnG/rhEGF/CS/Pol 407 hydrogel showed the ability to inhibit bacterial growth (*S. aureus* and *P. aeruginosa*) and biofilm formation ( $p < 0.05$ ). An *in vivo* study demonstrated that the hydrogel promoted burn wound healing in a rat model. ZnG influenced vascular remodelling, whereas rhEGF promoted fibrogenesis. The hydrogels were biocompatible, non-irritant and exhibited an anti-inflammatory property.

### 3.4. Chitosan Hydrogels with Fibroblast Growth Factors (FGF)

As FGFs bind to tyrosine kinase receptors (FGFR1-4), thus they take part in the mechanisms of wound healing and tissue repair. They stimulate cell proliferation, inhibit apoptosis, and induce angiogenesis and protease expression, which promotes wound healing [24]. They have a protective function against cellular stress [25]. FGF1, FGF2, FGF4, FGF7, FGF16, FGF21, and FGF23 have shown therapeutic effects in the treatment of diabetic foot ulcers [26]. Koike *et al.* [27] suggest that FGF2 may promote epithelial-mesenchymal transition (EMT) of keratinocytes, accelerating repair and regenerative processes in damaged tissues. No information was found on the adverse effects of FGF on wounds and tissues.

Wei *et al.* [14] developed a hydrogel based on chitosan (CS, deacetylation degree of  $\geq 95\%$ , viscosity of 100 - 200 mPa.s at  $T = 20^{\circ}\text{C}$ ) / alginate (SA) / poly(ethylene glycol) diacrylate (PEGDA) containing vascular endothelial cadherin (VE-cadherin) and fibroblast growth factors (FGF). The authors found that the amount of chitosan in the carrier affected the physicochemical parameters of the hydrogel. An increase in the amount of chitosan correlated with an increase in equilibrium bulk swelling due to a decrease in cross-linking density, leading to a lower  $G'$  (storage modulus) of the hydrogel. The hydrogel induced cell adhesion, proliferation and migration through PI3K/AKT signalling pathways. It had optimal haemostatic and antibacterial properties. The results obtained suggest antimicrobial properties of the formulation against *E. coli* and *S. aureus*. Histologically, better wound healing was observed in the full-thickness skin defect in a rat model ( $p < 0.05$ ). In a subsequent study, the same team [15] confirmed that VE-cadherin and FGF had a synergistic effect in the promotion of cell proliferation and migration ( $p < 0.05$ ). The hydrogel had an adequate pore size and optimal stability. It was

suggested that the chitosan hydrogel supports the healing of both superficial and deep burn wounds with necrotic tissue. The hydrogel promoted active cell growth for 12 days, and deep burn wounds healed within 24 days ( $p < 0.05$ ).

Liu *et al.* [16] developed a hydrogel consisting of chitosan (CS, molecular weight of 100 - 150 kDa, deacetylation degree of 85%), hyaluronic acid (HA), and gold nanoparticles (AuNP), into which fibroblast growth factors (FGF) were incorporated. The developed formulation showed antibacterial properties against MRSA (methicillin-resistant *S. aureus*), *S. aureus*, *E. coli*, and *P. aeruginosa*. The hydrogel was biocompatible with NIH/3T3-L1 and L929 fibroblast cell lines, and it mediated in promoting cell migration and proliferation. The high therapeutic potential of hydrogel in the treatment of diabetic wounds was suggested.

### **3.5. Development Stage of the Formulation in the View of the Literature**

An analysis of the scientific literature indicates that the current studies are at an early preformulation stage. However, it should be noted that there is also a single study in which a different polymeric substrate was employed, which may suggest potential directions for further clinical trials. The ClinicalTrials.gov database (official website of the U.S. Department of Health and Human Services, National Institutes of Health, National Library of Medicine, and National Center for Biotechnology Information) reports a completed clinical trial on the efficacy of a sodium carmellose-based hydrogel with becaplermin (Regranex Gel<sup>®</sup> with recombinant human platelet-derived growth factor BB) in the treatment of hypertensive leg ulcers. However, the efficacy of this preparation in human treatment was not confirmed in the study conducted.

## **4. Challenges in Developing Chitosan Hydrogel Carriers for Hormones and Hormone Derivatives Delivery**

Despite significant medical advances, chronic wound therapy remains a major challenge in clinical practice. A review of the literature indicates that chitosan-based hydrogels represent a promising biomaterial for the treatment of hard-to-heal wounds. On the other hand, systems of hybrid chitosan hydrogel with hormones show great potential in the development of epidermal therapy to promote the healing of chronic wounds. A major advantage of hormones or hormone derivatives is their high therapeutic efficacy at low doses. This also indicates a necessity for the precise determination of their doses in the hydrogel matrix [28]. Hormones and hormone derivatives do not convert to toxic metabolites and do not accumulate in the body. Nevertheless, the unfavourable wound microenvironment limits the topical use of hormones. The disruption or loss of the pharmacodynamic properties of hormones applied epicutaneously, which ultimately reduces the effectiveness of treatment, is the main obstruction. This is due in part to their high susceptibility to enzymatic degradation, as the amide bonds present in the hormone structure are subjected to proteolytic hydrolysis. Additional challenges are their limited penetration through the skin and their rapid removal by the exudate, containing, besides proteolytic enzymes, inflammatory mediators [29, 30]. Maintaining a stable spatial structure of hormones or hormone derivatives is critical to achieving a therapeutic effect.

### **4.1. Problem Aspects of the Formulation Process**

Prescription medicines are prepared in the pharmacy in accordance with the requirements set out in the European Pharmacopoeia (or other respective versions) using authorised raw materials and obeying good pharmacy practice (GPP). Particularly in Poland, the raw

materials have to be approved for trading by the President of the Office for Registration of Medicinal Products, Medical Devices and Biocidal Products, and prescription drugs are subject to quality testing in accordance with the provisions of the local pharmaceutical law [31]. The main regulatory and quality control challenges for chitosan-based hydrogel preparations with hormones or growth factors are the instability of the API, the use of stabilising excipients (often protected by patents), and the risk of immunological complications and adverse reactions, especially in patients with comorbidities. These issues require special considerations at the formulation development stage. It should be emphasised that safety and tolerability studies, especially in high-risk populations, are necessary before clinical use.

Chitosan as an excipient is included in the European Pharmacopeia 11.0 and the 29<sup>th</sup> edition of the United States Pharmacopeia (USP) 34-NF. However, there are no pharmacopoeial standards for chitosan-based hydrogels [32–34]. Therefore, it is important to understand the limitations of using a CS-based hydrogel matrix. In the technology of formulations encompassing chitosan, an organic acid environment is necessary to dissolve this biopolymer, which limits the possibility of low pH-sensitive active substances incorporation into the matrix [35]. Chitosan also comes in many variants, differing in the degree of deacetylation, molecular weight, which affects the reproducibility of its preparation technology and the quality of the resulting hydrogel [36]. In the context of further development of drug formulations with chitosan, we suggest undertaking research on the possibility of making hydrogels under acid-free conditions. Clinical trials should include an assessment of the formulation's stability under changing conditions in the wound environment and confirm its safety and efficacy.

Peptide hormones are sensitive to changes in pH, temperature, ionic strength, and pressure because their secondary, tertiary, and quaternary structures are stabilised by weak non-covalent bonds. Even a minor alteration of these parameters may result in the loss of hormone biological activity, which makes the technology for the preparation and storage of hormone drugs challenging. Some authors suggest that hydrogel carriers may be effective with respect to hormone stability, as their porous structure allows efficient incorporation of active substances. Moreover, their ability to adapt to changes in pH and temperature may protect peptide hormones from degradation under harsh environmental conditions [37–42]. However, it should be borne in mind that the sterilisation of hydrogels is difficult and time-consuming. Concerning the Polish Pharmacopoeia FP XII “Drugs prepared in pharmacy”, it is suggested that the shelf life of prescription hydrogels (without added preservatives) is at most 7 days [43]. However, determining the shelf life of a drug requires a detailed analysis of potential adverse changes that may occur during its use, as well as consideration of the expiry date of the pharmaceutical raw materials or finished preparations used.

## **5. Conclusions**

A review of the literature indicates that chitosan hydrogel carriers containing hormones or hormone derivatives have some potential in the treatment of hard-to-heal wounds. They are distinguished by favourable physicochemical properties, allowing epidermal application, as well as simplicity of preparation and low cost of production. They provide an effective carrier, ensuring optimal API stability. Depending on the composition, they can exhibit antimicrobial activity, promote angiogenesis, facilitate collagen deposition, and stimulate keratinocyte migration. However, it should be noted that the lack of a suitable chitosan raw material makes it impossible to make them under pharmacy

formulation conditions. In addition, the lack of clinical trials suggests the need for further studies on their efficacy and safety.

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