

POSSIBLE HYDROGEL BIOMATERIALS FOR THE TREATMENT OF DIFFICULT-TO-HEAL WOUNDS

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Abstract

According to the European Wound Management Association's TIME strategy (T – tissue management, I – infection and inflammation control, M – moisture balance, E – epithelial advancement), the modern concept for the topical treatment of difficult-to-heal wounds involves cleaning the wound, controlling infection and inflammation in the wound, providing adequate moisture and stimulating epithelial formation. Ensuring adequate moisture in the wound bed and supporting the self-renewal processes taking place in the wound are tasks in which modern, biologically active dressings play a key role. The introduction of hydrogel dressings modified with biologically active substances allows local, targeted delivery of these substances directly to the wound, which may improve the healing process. Hydrogel dressings based on natural ingredients can be adapted to different types of wounds and individual patient needs, thereby increasing their effectiveness. Modern biologically active hydrogel dressings containing bioactive substances are an alternative to dry dressings and can effectively improve the comfort of wound treatment.

Keywords: hydrogels, alginates, bioactive substances

Introduction

Lifestyle diseases, including cardiovascular diseases, obesity, diabetes and cancer, cause approximately 40 million deaths each year, accounting for about 70% of all deaths worldwide. At present, more than 2 million people in Poland have diabetes, approximately 25% of whom are unaware of the disease. Forecasts indicate that the number of people with diabetes in Poland will double over the next 15–20 years. It is worth noting that the lifetime risk of ulceration in a person with diabetes is 12–25%, while the probability of amputation in diabetic patients is 30–40 times higher than in people without diabetes. The global population is ageing rapidly. The number of people affected by obesity and diabetes is also increasing, resulting in a marked rise in the prevalence of chronic wounds. It is therefore extremely important to de-

velop biomaterials that positively affect the difficult wound-healing process while keeping costs as low as possible so that dressings are accessible to everyone (Gupta et al., 2019; Salehi et al., 2021). The development of innovative wound dressings with healing-accelerating properties has recently become the subject of extensive research (Wang et al., 2022). The prospect of producing a hydrogel dressing containing natural ingredients, such as sheep or camel milk or plant substances rich in natural bioactive compounds, is highly promising. This represents a major opportunity to improve treatment comfort for patients with burn wounds or diabetic wounds.

Wound healing is a complex biological process consisting of a series of successive biochemical reactions. Four phases of tissue repair can be distinguished: the exudative phase, limitation of inflammation and wound cleansing, the proliferative phase, and the remodelling phase (Fig. 1).

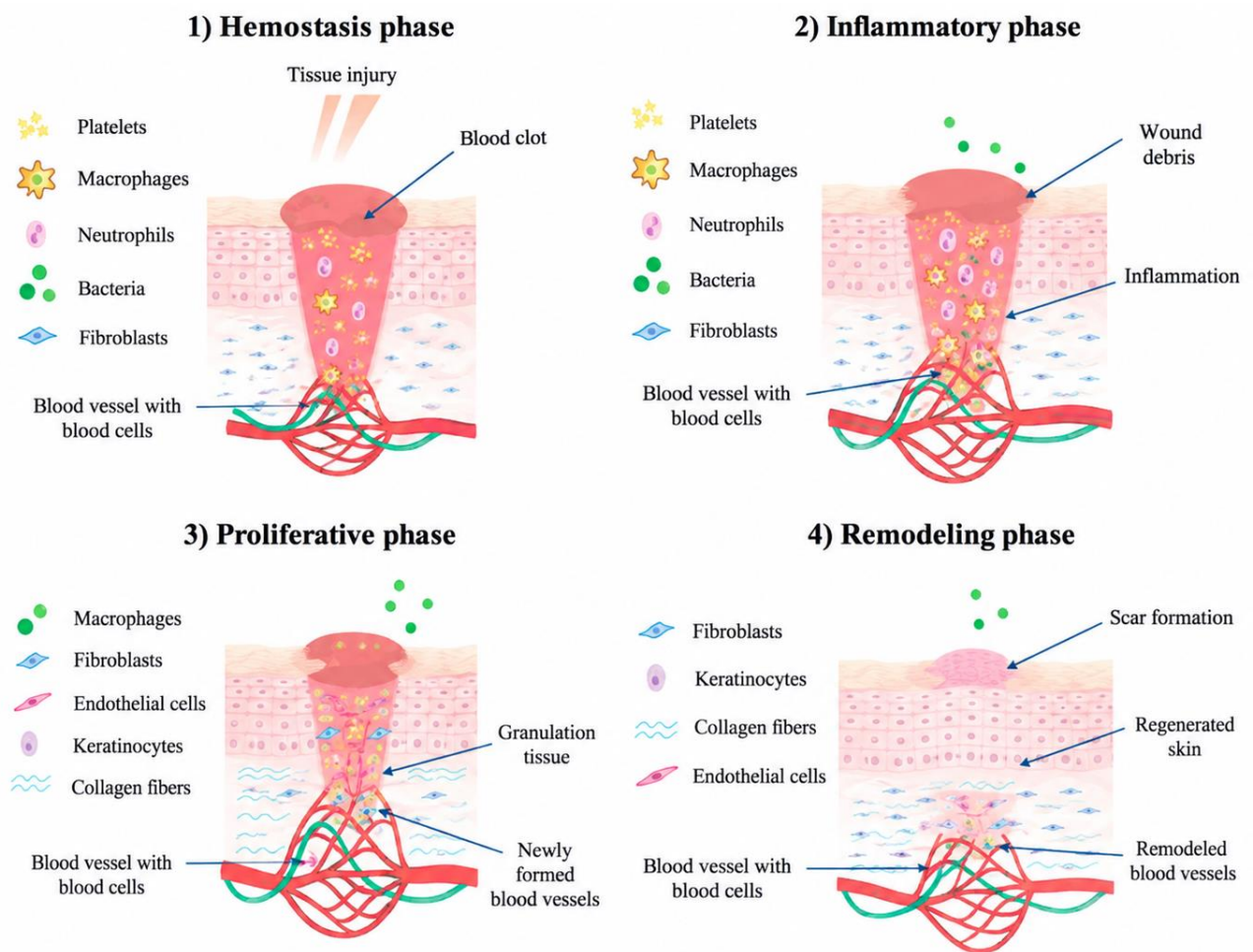


Fig. 1. Stages of the wound healing process (Gupta et al., 2019, as modified by P. Szatkowski)

Normal, healthy wound healing follows the stages described above; however, the orderly sequence of this process is very often disrupted, resulting in abnormal and prolonged treatment. Wound healing is influenced by local factors, such as tissue oxygen tension, infection and pain, as well as systemic factors related to the patient's health, including diseases or therapies that weaken the immune response. Infected wounds usually remain arrested in the inflammatory phase, so the process no longer follows the normal healing pattern. Wound

cleansing is therefore extremely important. Excessive drying of the wound may also slow granulation and epithelial formation. Further important factors are wound injuries that destroy newly formed delicate tissue. Excessive exudate may inhibit cell proliferation and degrade surrounding tissue because it contains high concentrations of cytokines (Koehler et al., 2017). Chronic non-healing wounds occur in all age groups but are most frequently observed in older people (Gupta et al., 2019).

Traditional wound dressings, such as gauze, adhesive dressings or cotton bandages, provide a barrier between the wound and the external environment, protecting the body from contamination and bacterial infection. They absorb a large proportion of the moisture released from the wound, which is desirable for proper healing. However, these dressings can excessively dry the wound surface, slowing healing and potentially causing pain during dressing changes. Appropriate material selection is crucial for normal wound healing. Traditional dressings are suitable for clean, dry wounds with mild exudate because they do not provide a moist environment. More advanced dressings with modern formulations should be used for other types of difficult-to-heal wounds (Dhivya et al., 2015; Rezvani Ghomi et al., 2019).

The concept of an ideal dressing

Wet dressings based on hydrogels accelerate wound healing compared with dry dressings. An ideal dressing should control moisture around the wound. By creating a moist environment, skin renewal can occur without inflammation and without scab formation. This can be achieved using hydrophilic materials capable of binding and absorbing water (Rezvani Ghomi et al., 2019).

Another important aspect is the appropriate porosity of the material used to produce the dressing. A porous scaffold promotes skin-cell proliferation and facilitates cell propagation. In addition, porous materials provide excellent gas transmission and nutrient diffusion. Studies have shown that the mean pore size required for wound-healing applications should range from 20 μm to 135 μm (Salehi et al., 2021). In addition to porosity, the substances used to form a bioactive dressing influence cell proliferation, growth and viability. Alginate, collagen, hyaluronic acid, polyethylene glycol, chitosan and poly(vinyl alcohol) are widely used dressing components; they provide satisfactory results and positively affect the rate of skin healing. These polymers are used alone or in combination depending on the nature and type of wound. Collagen initiates fibroblast formation and accelerates endothelial migration in contact with damaged tissue. Hyaluronic acid (HA) is a glycosaminoglycan component of the extracellular matrix (ECM) with unique biological and physicochemical properties. Like collagen, HA is biocompatible, biodegradable and naturally non-immunogenic. Chitosan promotes granulation tissue formation during the proliferative stage of wound healing. Biological dressings are non-toxic, biocompatible and biodegradable (Dhivya et al., 2015; Gupta et al., 2019; Khan et al., 2020; Salehi et al., 2021).

A dressing forms a barrier between damaged epidermis and the external environment and provides mechanical protection. Microorganisms are an external factor that can disrupt wound healing, arrest the process and cause infection. Incorporating therapeutic agents into hydrogel dressings enables faster and more effective epidermal regeneration. Materials currently used in dressings for therapeutic-agent delivery include hydrocolloids, hydrogels, films and polyurethane foams. An ideal dressing material should contain a bactericidal agent. Bacterial cellulose, graphene oxide and silver nanoparticles are often used for this purpose (Dhivya et al., 2015). In recent years, plant-derived substances such as curcumin from turmeric rhizomes and

naringenin, a compound present in grapefruit juice, have also become popular (Gupta et al., 2019; Salehi et al., 2021).

Chronic wounds are burdensome and extremely painful for patients. In addition to a moist dressing that relieves pain and provides a cooling sensation, incorporating a therapeutic substance can provide analgesic and anti-inflammatory effects. Selected dressing components should respond to stresses, for example those caused by impact, by neutralising their force so that the wound remains intact and the healing mechanism proceeds without disturbance (Dhivya et al., 2015). The material should be easy to replace or remove and should remain on the wound for as long as possible while maintaining a moist environment. Transparent dressings are therefore advantageous because they permit monitoring of healing. Dressings containing a pH indicator may also be useful. The pH profiles of healthy skin, acute wounds and chronic wounds differ considerably. Chronic wounds have an alkaline pH, whereas healthy skin is slightly acidic, approximately 4.7–5.6. Chronic wounds heal more slowly than acute wounds with a neutral pH. Neutral pH is associated with bacteria proliferating in wounds and preferring a pH around 7. A pH indicator can cause the dressing to change colour, enabling a rapid response and appropriate treatment. Following this reasoning, an ideal 'smart' dressing would lower the pH of damaged skin to that of healthy tissue and reduce its temperature, which rises during inflammation, to the normal epidermal range, thereby creating less favourable conditions for bacterial growth (Jones et al., 2015).

Polymer hydrogels

Hydrogels are cross-linked hydrophilic polymer networks with a high water content. They are highly versatile because their chemical and physical properties can be manipulated, making it possible to design a gel best suited to a particular application. The swelling capacity of hydrogels allows diffusion of molecules and cells, while their similarity to natural soft tissue is also attractive for biomedical applications. Hydrogels can be obtained from naturally derived polymers, such as polysaccharides, or from synthetic polymers, such as polyacrylates (Dhivya et al., 2015). Materials based on natural polymers are characterised by biodegradability, biocompatibility with various body tissues, low cytotoxicity and similarity to the physiological environment of the human body. They can therefore play an important role in the medical applications described above (El-Sherbiny and Yacoub, 2013), although they also have limitations, particularly weak mechanical properties. For this reason, natural hydrogels are often modified to form composite polymers.

Hydrogels have been extensively studied for biomedical applications including drug-delivery systems, 3D cultures, tissue implants, tissue regeneration, contact lenses and dressings for difficult-to-heal wounds. Each hydrogel type can be adapted to a specific purpose, and hydrogels are therefore produced using various techniques. Research aimed at developing hydrogels for biomedical applications is one of the most intensively studied areas at the interface of engineering and medicine (Mandal et al., 2020). Hydrogels can be classified according to the interactions between polymer chains, the origin of the polymer used to obtain the hydrogel, and their sensitivity and response to environmental stimuli (Fig. 2).

Interactions between hydrogel components	Polymer origin	Sensitivity and response to environmental stimuli
• Physical • Chemical	• Synthetic • Natural	• Change in pH • Change in temperature • Application of an electric field

Fig. 2. Classification of hydrogels

Depending on the type of interactions between hydrogel components, hydrogels are divided into chemical and physical types. Chemical hydrogels are characterised by strong and permanent covalent bonds and therefore have better mechanical properties and greater stability under physiological conditions than physical hydrogels. In physical hydrogels, polymer chains are linked by ionic and hydrogen bonds and van der Waals interactions. Their principal advantage is that chemical cross-linking agents are not required during preparation. Consequently, these materials have low toxicity and are frequently used in biomedical applications (Hu et al., 2019).

Water is an extremely important hydrogel component that affects its properties and, to a large extent, its potential use as a broadly defined therapeutic agent. The ability of hydrogels to swell, i.e. to absorb water, is particularly important when they are used as dressings. Water absorption is associated with hydrophilic functional groups in the polymer structure, including amino (NH_2), hydroxyl ($-\text{OH}$), amide ($-\text{CONH}-$, $-\text{CONH}_2$) and sulfate ($-\text{SO}_3\text{H}$) groups. Because of these hydrophilic groups, water molecules can migrate into the polymer network, causing hydrogel expansion and an increase in volume (El-Sherbiny and Yacoub, 2013). Their adjustable structure, manipulable properties, natural flexibility, excellent biocompatibility, physiologically similar environment and dynamic mechanical strength make hydrogels ideal materials for biomedical applications.

Characteristics of selected substances for hydrogel production

Gelling polymers

Natural polymers are very frequently used in biomedical applications. They can be defined as materials commonly occurring in nature, originating from many different sources and extracted from plants or animal material. These polymers are, or can be, biocompatible, non-toxic and biodegradable, making them excellent materials for biotherapeutic applications. One such polymer is sodium alginate, which occurs in brown algae and in the capsules of bacteria such as *Azotobacter* and *Pseudomonas*. Alginates consist of a linear anionic polysaccharide polymer of β -(1-4)-D-mannuronic acid (M blocks) and α -L-guluronic acid (G blocks) (Fig. 3).

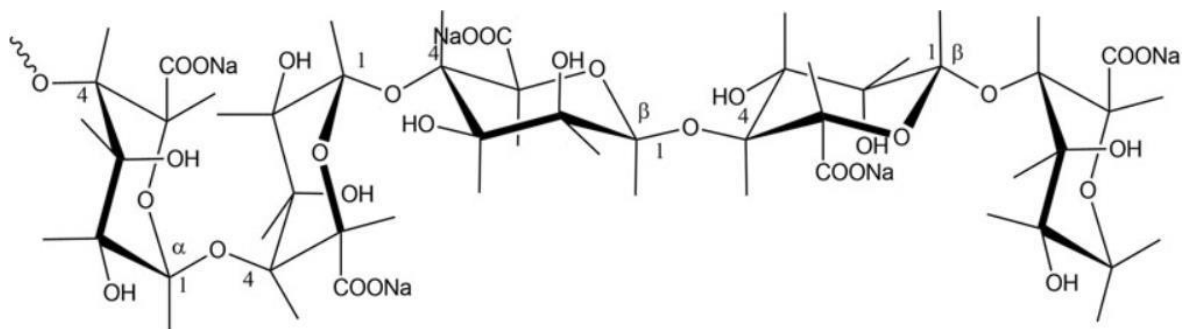


Fig. 3. Structure of sodium alginate (Mollah et al., 2021)

Alginate exhibits gelling properties because of the abundance of GM blocks and their interchain interactions. Alginate gels are widely used in the food, textile and paper industries as well as in medicine. Alginate has great potential for biomedical applications because of its biodegradability and biocompatibility. It is used as a dressing for acute or chronic wounds and as a scaffold for tissue regeneration. Alginate extracted from *L. hyperborea* contains 60% G blocks, whereas alginates from other commercial algal species contain 14–31% G blocks. Only the carboxylate groups of G residues have been shown to cross-link with divalent cations such as Ca^{2+} and Mg^{2+} to form a hydrogel. Thus, alginates with a high G-block content

form stiffer hydrogels, whereas alginates rich in M blocks gel into softer, more flexible structures. The softer structure of an alginate hydrogel limits its potential for soft-tissue regeneration and makes it unsuitable for load-bearing parts of the body. To address this problem, various natural and synthetic substances are added to the alginate structure to form stronger composite materials. Alginate composites also improve drug-release capacity and cell viability. Moreover, hydrogels can be produced relatively easily and inexpensively, and an appropriate cross-linking method can yield a material with excellent properties (Mollah et al., 2021). Wound dressings prepared from alginate can maintain a moist environment, absorb excess wound fluid and minimise bacterial infection at the site of tissue damage.

Another polymer used to produce hydrogels is poly(ethylene glycol) diacrylate. Polyethylene glycol (PEG)-based polymers are used in various biomedical applications because they can be readily synthesised, show good biocompatibility and possess tissue-like properties. Poly(ethylene glycol) diacrylate (PEGDA) contains acrylate groups with a double bond at each end of the PEG chain (McAvoy et al., 2018). PEGDA is biocompatible with many tissues, non-toxic, biodegradable and resistant to protein adsorption. PEG-based materials show high moisture adsorption because of hydrogen bonding between the PEG chain and water molecules. PEGDA is advantageous for biological applications and is used in the engineering of many tissues, including bone, cartilage and cornea (McAvoy et al., 2018; Nadhif et al., 2021).

Ginger, sheep milk and camel milk

Hydrogel dressings can be supplemented with modifying substances that alter their physical properties and mechanical stability. Such substances may also affect resistance to high temperatures and control degradation. Moreover, the modifying substance itself may have bioactive properties. Bioactivity is an essential feature of modern materials intended for biotherapeutic applications. In the context of difficult-to-heal wounds, bioactivity should involve the release of substances that support individual stages of healing. Selection of an appropriate bioactive modifier often determines the success of a material used in bioengineering. One plant-derived substance used in biomedical materials is ginger extract. Ginger rhizomes naturally contain polyphenols, a common group of antioxidants characterised by phenolic groups in their structure. Polyphenols are highly diverse: more than 8,000 phenolic compounds are currently known, including 5,000 flavonoids. These biomolecules have anticancer, anti-atherosclerotic, hepatoprotective and antiallergic properties. Numerous health benefits of ginger, including antidiabetic effects, have been demonstrated (Kayath et al., 2020; Mashhadi et al., 2013). Other studies have shown that ethanolic ginger (*Zingiber officinale*) extract supports wound healing by promoting collagen deposition, wound contraction and re-epithelialisation (Khan et al., 2020).

In recent years, particular attention has also been paid to sheep and camel milk as components of hydrogel biomaterials. Their high contents of health-promoting bioactive substances confer special properties on these products. Sezik et al. (2001) reported that sheep milk has been used in traditional medicine to treat difficult-to-heal wounds, bites and purulent skin lesions. Poor diet, repeated injury and diabetes are among the factors that severely disrupt wound healing. Depending on wound size and the extent of damage, extensive physiological and metabolic changes may occur, causing numerous complications in healing and reducing patient immunity (Hrynyk and Neufeld, 2014). In the era of increasingly prevalent lifestyle and metabolic diseases, allergies and cancers, it is therefore important to support the body with natural products having specific therapeutic properties. Sheep and camel milk belong to this group because of their rich profiles of bioactive substances. Compared with cow's milk, both contain high levels of insulin, which is particularly important in wound healing. Insulin regulates blood glucose and may support healing of damaged skin by regulating oxidative and

inflammatory responses and stimulating cell migration. Studies indicate that topical insulin administration significantly affects healing of diabetic, burn, acute and chronic wounds. In diabetic patients, local insulin injection at the wound site promotes healing by improving granulation-tissue growth (Zhang and Lv, 2016). However, injection into sensitive tissue is painful and difficult for patients to perform themselves, while the effectiveness of absorption from medical insulin creams may be reduced when the preparation is rubbed off the wound surface. Integrating insulin into hydrogel dressings may therefore be a breakthrough because of the ease of application and use. Studies have shown that incorporating insulin and fibroblasts into a hydrogel dressing may promote neovascularisation and collagen deposition and improve diabetic wound healing (Zhao et al., 2017). Biomaterials used as wound dressings have attracted increasing attention in recent years, and hydrogels loaded with bioactive substances have potential as targeted therapeutic systems (Zhao et al., 2017).

Lactoferrin (LF) is an iron-binding glycoprotein that supports many biological processes involved in wound healing. It has anti-inflammatory activity, directly promotes granulation-tissue formation, stimulates proliferation and migration of fibroblasts and keratinocytes, and enhances collagen and hyaluronan synthesis. Because of its high lactoferrin content, sheep milk may reduce autoimmune inflammatory processes and provide protection against bacterial and viral infections (Caboni et al., 2019). Camel milk is rich in bioactive substances such as active peptides, LF, zinc, and mono- and polyunsaturated fatty acids. Consequently, camel milk also has antioxidant, antimicrobial, antidiabetic and cholesterol-lowering effects. LF content is 0.2–0.9 g/kg in camel milk, 0.7–0.9 g/kg in sheep milk and 0.02–0.5 g/kg in cow's milk (Alichanidis et al., 2016).

Introducing natural bioactive substances into hydrogel dressings may accelerate wound healing. The camel-milk fat globule membrane has anti-adhesive and antibacterial properties. Mansour et al. (2015) reported that a cream containing 25% sheep milk fat globules accelerates burn healing and reduces inflammation, promotes wound contraction, minimises scarring and improves burn-wound treatment. Other lipids with important biological roles include butyric acid, conjugated linoleic acid (CLA), sphingolipids (components of fat-globule membranes), and carriers of vitamins A, D, E and K and carotenoids. The most biologically active CLA isomer is cis-9, trans-11, which inhibits the occurrence and development of skin cancer (Wang and Jones, 2004). One specific feature of camel milk is the presence of CLA, although among ruminants sheep milk contains the highest amount of CLA (Molik et al., 2020). Park et al. (2010) showed that CLA supplementation accelerates skin-wound healing by regulating antioxidant and anti-inflammatory functions. This creates an opportunity to investigate the topical effect of CLA on wound healing.

Summary

Hydrogel dressings act not only as mechanisms for releasing regenerative factors but also as scaffolds for epidermal-cell proliferation, providing both chemical and mechanical cues. Hydrogel materials create a moist wound environment that improves typical stages of healing, including granulation-tissue growth, epidermal repair and removal of excess necrotic tissue. The limited adhesion and moisture of hydrogels allow dressings to be removed easily without secondary trauma, considerably reducing discomfort and the risk of infection associated with dressing changes. A moist dressing also has a cooling effect that reduces pain. Some hydrogel dressings can be designed to be transparent, allowing clinical assessment of wound healing without removing the dressing and thus avoiding trauma caused by dressing changes. A hydrogel dressing can also be designed to respond to changing pH, which is extremely important for monitoring the stages of wound healing. Another important parameter in supporting the

treatment of skin wounds is the dressing's ability to absorb wound exudate. The development of innovative wound dressings with healing-accelerating properties has recently become the subject of extensive research. Hydrogel dressings can be adapted to different types of wounds and individual patient needs, thereby increasing their effectiveness. From an economic perspective, a targeted innovative dressing that accelerates healing and reduces the risk of complications may contribute to lower healthcare costs. Hydrogel dressings can deliver bioactive substances more precisely and effectively. In addition, they can be freely modified to meet individual patient needs because the active substance is directed straight to the wound.

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