Natural Polysaccharides on Wound Healing 46

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Abstract

As a result of diseases and accidents, people lose their tissues and organs. Instead of difficult and troublesome methods such as tissue and organ transplantation, biocompatible, nontoxic, antitumor, antimicrobial, and wound healing natural polymers are used for the treatment of these damages. There are glycosaminoglycans as natural polysaccharides in the human body, which act as extracellular matrix and produced from fibroblasts. Wound healing is a dynamic and complex process consisting of successive periods. Tissue healing process is regular and timely in acute wounds. In chronic wounds, healing takes longer time. The use of appropriate dressings plays an important role in the wound healing process. Researches on polymeric dressings used as carriers for

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local application of active ingredients to the wound surface are increasing. These polymeric systems can be natural, hydrogel-forming materials such as collagen, chitosan, and pectin, or tissue-engineered materials such as alginate. In this section, extracellular matrix, wound formation, wound healing mechanisms, and the function of natural polysaccharides which have an important role in wound healing will be examined.

Keywords

Tissue · Extracellular matrix · Glycosaminoglycan · Polymer · Wound healing

1 Introduction

Wound healing is one of the most common health problems and advanced wound management strategies are needed to achieve optimal healing. Definite times cannot be given for wound healing, but the chronic term is used for wounds that cannot heal within a reasonable time. The progression of wound healing depends on many factors, ranging from the patient's general condition to treatment, to the cause. It is necessary to act according to the characteristics of each wound. Wound healing depends on various factors such as the general health of the person and blood circulation, the reason for the formation of the wound, the location and type of wound in the body, and whether there are infections in the wound. So every wound heals according to its own characteristics (Schreiber et al. 2005).

Wounds are divided into two parts as acute and chronic wounds according to the wound healing time. Acute wounds are wounds that we encounter very often, do not cause damage after recovery, and heal quickly. Chronic wounds, on the other hand, are characterized by impaired skin integrity, functional loss, and prolonged recovery. Recovery in chronic wounds takes weeks, months, and years. In this context, appropriate wound treatment is required, which can speed up the healing process and reduce the healing time at the same time. Glycosaminoglycan derivatives, which are mostly involved in the structure of the extracellular matrix (ECM) in wound healing, have been preferred as potential treatments for many years (Tan et al. 2001).

Glycosaminoglycans (GAGs), the extracellular matrix molecule, which plays an important role in the acute or chronic wound healing process and supports wound healing, is an effective means of angiogenesis and inflammation and leads to rapid granulation, vascularization, and re-epithelization (Mitchell and Church 2002). Glycosaminoglycans are preferred in many materials due to their function in wound healing and are recommended to reduce the healing time in the chronic wound healing process. In this section, the natural polysaccharides used in wound healing and their mechanisms will be discussed in detail.

2 Extracellular Matrix and Wound

2.1 Extracellular Matrix

Connective tissue epithelium, which protects the integrity of the body and keeps all parts of the body together structurally and functionally, provides continuity between the muscle and nerve tissues. Connective tissue covers all the tissues that support the structure of the body, such as ligaments of the joints, beams, bone tissue, cartilage tissue, and adipose tissue.

The intercellular substance (extracellular matrix/extracellular matrix) created by connective tissue cells consists of two main structures:

1. Connective tissue fibers.
2. The basic substance that fills the space between connective tissue fibers and connective tissue cells.

Basic substance: It is the basic structure consisting of proteoglycan, glycoprotein, and glycosaminoglycans, and forming the extracellular space together with cell fibers. This structure forms the basic environment that meets the actual needs of the cell and tissue (Baum and Arpey 2005).

Extracellular matrix (ECM), which plays a role in the connective tissue as an extracellular matrix, is a variety of proteins and polysaccharides that are secreted by some cells in a multicellular organism, which fill cells between cells and act as binding agents in a defined area (Karabekian et al. 2009). There are two main extracellular proteins that make up the matrix. These are fibrous and proteoglycans (Ustunel et al. 2003).

Proteoglycans are peptide chains containing covalently linked glycosaminoglycans. They contain 95% carbohydrates and 5% protein in their structure. There are seven types of glycosaminoglycans (GAGs): hyaluronic acid, chondroitin sulfate, keratan sulfate I and II, heparin, heparan sulfate, and dermatan sulfate. Fibrous proteins are two types: structural proteins (collagen and elastin) and adhesive proteins (fibronectin, laminin, tenaskin, vitronectin, and integrin) (Wound repair 2005).

Glycosaminoglycans form a gelatinous and hydrated substance in the connective tissue by proteoglycans (PGs) by embedding fibrous proteins. Proteoglycans consist of a central protein called glycosaminoglycans (GAGs) that are bound to one or more polysaccharides (Pelosi et al. 2007). Glycosaminoglycans are heterogeneous polysaccharides containing long, linear, and recurrent disaccharide units. These disaccharide units are galactose, galactosamine, N-acetylgalactosamine-4-sulfate, and galacturonic acid. There are two basic types of GAG. The first one is non-sulfated GAG (hyaluronic acid), and the second is sulfated GAG (heparan sulfate, heparin, chondroitin sulfate, dermatan sulfate, and keratan sulfate). Except for hyaluronic acid, GAGs are usually covalently attached to a protein nucleus that forms a general structure called proteoglycans (Pelosi et al. 2007; Souza-Fernandes et al. 2006).

GAGs are usually large complexes consisting of small amounts of protein and negatively charged heteropolysaccharide chains. These complexes form a gel-like matrix called "ground substance" with a large amount of water binding property. They are also strong acidic biopolymers with biomedical importance that prevent the formation of factors such as virus entry into cells and angiogenesis. GAG chains fill most extracellular spaces and provide mechanical support to the tissue, as well as providing rapid diffusion of water-soluble molecules and migration of cells (Ramael et al. 1991).

2.2 Wound Formation: Acute and Chronic Wound

The wound is the disruption of the integrity of the skin or mucosa due to the effect of trauma. Factors such as shock, falling, strong impacts, atmospheric pressure, thermal effects, (burn, freezing) electric shock, and radioactivity cause injuries. As a result of acute or chronic wound, tissue integrity is impaired. Acute wounds heal without problems within the expected time. The time it takes for chronic wounds to heal usually ranges from 5, 10, or 30 days. It may occur as a result of traumatic loss of tissue or surgical procedure (Jackson et al. 1991).

Chronic wounds are often characterized by permanent injury and prolonged inflammation, high bacterial biofilm incidence, and excessive proteolysis (Robson et al. 2001). Impairment in macrophage function and angiogenic response, which is often associated with serious wound healing process, is also observed. Due to prolonged inflammation, excessive removal of inflammatory cells into the wound bed by a large number of neutrophils is required. It is known that neutrophils can remove damaged tissue from the temporary matrix of the wound site and prevent microbial infection. On the other hand, as the potential of unmanaged neutrophils to kill pathogens causes degradation of the ECM and growth factors, it can cause excessive production of protease that initiates significant tissue damage to the host that is detrimental to wound healing. In addition, inefficient cell proliferation due to the breakdown of the ECM molecule in the wound leads to angiogenesis, which indicates greater wound bed defacement and healing impairment. Therefore, prevention of prolonged inflammation is a target strategy in the treatment of severe wounds. GAG has been found to bind to neutrophils, macrophages, and lymphocytes, key cells of the inflammatory response. The effect of excessive protease production caused by too many active neutrophils in the wound area can be inhibited by electrostatic binding with some anionic polymers, such as GAG or functionalized dextrans. A high level of anionic GAG will be in ion pairing with cationic neutrophils to interfere with the activity of cationic proteins through charge interactions. Therefore, with this mechanism, it may be possible to reduce excessive neutrophil uptake and move the wound from the inflammatory stage to the next healing stage. However, after severe tissue damage, glycanases and proteases can destroy GAG (Komarcevic 2000).

In severe wounds, GAG deficiency is eliminated by adding GAG material, such as a natural polysaccharide, directly to the wound area as a wound dressing. With the rich source of GAG around the wound and a better understanding of the GAG roles in the healing processes, it was possible to formulate therapeutic strategies expected

to accelerate serious wound healing. Glycosaminoglycans (GAGs) have been shown to play important roles in cell signaling and development, angiogenesis, anti-coagulation, and co-receptors for growth factors that belong to control of all wound healing stages, both acute wounds and chronic wounds (Peplow 2005).

In chronic wounds, delays occur in the normal stages of healing, the wound cannot be repaired regularly and on time (Jackson et al. 1991). As a result of disruptions caused by various factors in one or more stages of hemostasis, inflammation, proliferation, or remodeling, the healing process cannot be completed completely. This may be caused by increased levels of infection, tissue hypoxia, necrosis, exudate, or inflammatory cytokines. Continued inflammation in the wound causes the tissue to heal response to occur in an uncoordinated and long period, resulting in frequent recurrence of wounds. Chronic wounds can be caused by various causes such as pressure, arterial and venous insufficiency, burns, continuous infection, and vasculitis (Jackson et al. 1991; Komarcevic 2000).

Among the various molecules secreted by ECM, GAG has partners that have important roles in controlling all wound healing stages, acute wounds or severe wounds. These molecules participate in cell-cell and cell-matrix interactions, cell proliferation and displacement, cytokine and growth factor signals, thereby locally modulating their biological activities. The ECM functions to guide the organized response characterized by hemostasis, inflammation, proliferation, and restructuring seen in wound healing. The effects of the various ECM components differ according to the wound stages. This dynamic and sequential order occurs as a result of the interaction of cell and growth factors (Ono et al. 1995).

3 Wound Healing

Wound healing is an extremely dynamic process and involves complex interactions of extracellular matrix molecules, soluble mediator molecules, various resident cells, and infiltrating leukocyte subtypes. The main purpose of the repair is to achieve tissue integrity and hemostasis. For this purpose, the healing process takes place in three stages (Negut et al. 2018).

1. Inflammatory phase (hemostasis and inflammation).
2. Proliferation.
3. Maturation and restructuring (remodeling).

In one of these phases, delay or negativity results in the wound not closing or healing is prolonged (Muncaster 2001).

3.1 Hemostasis

The first stage of wound healing is devoted to the formation of a hemostasis and a temporary wound matrix. The wound matrix appears immediately after the wound

and is completed a few hours later. This phase initiates the inflammatory process and is sometimes called the late phase (Robson et al. 2001).

The vital agents of hemostasis are fibrin, platelets, and blood vessels. In the first 1 or 2 h after injury, wound repair begins with the formation of a fibrin matrix through proteolytic cleavage of fibrinogen with thrombin, and fibrin binds directly to platelets to produce a clot (Muncaster 2001; Woo et al. 2004). This causes degranulation of alpha granules and dense bodies in the cytoplasm of platelets. In this way, albumin, fibrinogen, fibronectin, IgG, coagulation factor V and VIII, platelet-derived growth factor (PDGF), transforming growth factor alpha and beta, fibroblast growth factor-2 (FGF-2), platelet-derived epidermal growth factor (PDEGF), and endothelial growth factor are secreted into the environment. Among all these factors, PDGF, TGF-beta, and FGF-2 are the most important. Dense bodies, by releasing calcium, serotonin, ADP, and ATP, provide an energy source for inflammatory cells that will come to the environment (Monaco and Lawrence 2003). It also calls and activates PDGF and IGF-1 fibroblasts. GAG and collagen are synthesized to allow cells to migrate and multiply at the wound site. Fibrinolytic enzymes resist clot formation. On the other hand, sprinkles ensure that excessive fibrinolytic activity does not occur. The ECM includes a network of scaffolding proteins that are bound by GAG. GAG, especially heparin sulfate (HS), plays a key role as anticoagulants, which have important actions to manage the regulation of protein networks. HS represents 50–90% of the total GAG content and is only in contact with blood when an injury occurs. It has been determined that HS binds with more than 100 proteins involved in hemostasis, many growth factors, proteins involved in lipid metabolism, and ECM proteins. In addition, HS maintains hemostasis as an effective mediator of angiogenesis on the surface of endothelial cells (Jacob et al. 2007).

In this phase, many cells and factors are activated for the wound healing process occurring in the organism.

3.2 Inflammation

The main purpose of this phase in wound healing is to prevent infections. At this stage, the first response is to neutralize bacteria and foreign particles with phagocytosis or toxic substances released by the neutrophils coming into the scar tissue within 48 h (Lesko and Majka 2008). This phase occurs with symptoms of inflammation, such as redness, body temperature, swelling and pain around the injured place, on average, within 24–48 h. When bleeding is controlled, neutrophils, macrophages, and lymphocytes accumulate in the wound area, simultaneously releasing a large number of active mediators (cytokines and growth factors), thereby stimulating the inflammatory phase (Broughton 2nd et al. 2006; Gosain and DiPietro 2004).

Chemotactic agents such as increased vascular permeability caused by inflammation, complement factors, interleukin-1, tumor necrosis factor-alpha (TNF-α), tumor necrosis factor-beta, and platelet factor 4 (PF4) stimulate neutrophil chemotaxis (Broughton 2nd et al. 2006; van Beurden et al. 2005). Neutrophils appear in the

wound 6 h after trauma and are the cells that prevail for the first 2 days. The main task of neutrophils on the wound surface is to remove the traces of the cell that are damaged by trauma, by the phagocytosis of the foreign body, and the release of proteases (Monaco and Lawrence 2003).

Monocyte density begins on the second-third days. As the neutrophil count decreases, the number of monocytes/macrophages increases. In the third-fifth days, macrophages become 12 dominant cells in the wound (Gosain and DiPietro 2004). The presence of active macrophages in the wound area is essential for wound healing. While neutrophil absence does not disrupt the general flow of wound healing, wound healing process stops in the absence of macrophage. The main duties of macrophages in wound healing are outlined: phagocytosis and antimicrobial function, wound debridement, matrix synthesis regulation, cell activation, and angiogenesis (Broughton 2nd et al. 2006).

Macrophages not only do phagocytosis but also perform various cytokines, growth factors, and NO synthesis. They increase keratinocyte and fibroblast activation. In addition, while it catalyzes the conversion of plasminogen to plasmid with the help of neutral proteases, it also activates the complement and pre-Hageman factor (Lesko and Majka 2008). Activated macrophages are cells that play a key role in wound healing (Lesko and Majka 2008; van Beurden et al. 2005).

Hyaluronic acid (HA), a non-sulfated GAG of ECM, is involved in an important process of the inflammatory phase. At this stage, HA accumulates in the wound bed and regulates early inflammation to modulate inflammatory cell and fibroblast cell migration, pro-inflammatory cytokine synthesis, and phagocytosis of invading microbes. In addition, HA can bind and increase the effectiveness of chemokines to neutrophils. Butler et al. found that the efficiency of HA and neutrophil uptake on the endothelial surface was increased and revealed that HA revealed stimuli to neutrophils (Butler et al. 2009).

In the inflammatory phase, neutrophils collagenase and elastase eliminate damaged tissue from the temporary matrix of the wound site, while monocytes inactivate any source of microbial infections through macrophages and the activity of secreted proteases. In inflammation sites, low molecular weight HA fragments (accumulated by the degradation of high molecular weight HA) proliferate IL-6, TNF-α, and IL-1β of Toll-like receptor 2 and Toll-like receptor 4 pro-inflammatory cytokines. Also, growth factors and cytokines released by inflammatory cells induce migration and proliferation of fibroblast and keratinocyte, which synthesize HA levels (Zhong et al. 2010).

It was found that the level of HA was significantly high during the reepithelialization process, where epithelial cells migrated through the new tissue to form a barrier between the wound and the environment. The secretion of cytokines such as TGF-β, PDGF, FGF-2, IL-1, and TNF-α modulates collagen accumulation and penetration of new blood vessels into the wound area by fibroblasts. T lymphocytes (especially CD4) migrate to the wound area; it secretes IL-1, IL-2, TNF-alpha, fibroblast activating factor, EGF, and TGF-beta. Inhibition of circulating T lymphocytes delays wound healing. B lymphocytes have not been found to play a role in wound healing (Winter and Scales 1963).

In summary, in inflammation, leukocytes bind to ECM proteins through integrins. ECM proteins stimulate the activity of monocytes/macrophages and remove neutrophils and wound residues from the wound site (Miller et al. 1998).

3.3 Proliferation

This complex process consists of angiogenesis, granulation tissue formation, collagen deposition, epithelization, and wound closure, and takes about 2 days–2 weeks. A new matrix layer by fibroblasts restores tissue in the wound area. Other mesenchymal cells also enter the inflammatory region of the wound in response to growth factors necessary to stimulate cell proliferation (Lesko and Majka 2008). Also, fibroblasts, endothelial cells, and keratinocytes produce IGF-I, FGF-2, TGF-y, PDGF, and VEGF, which support cell migration and proliferation, matrix synthesis, and angiogenesis. Secreted vascular endothelial growth factor (VEGF) and other cytokines neovascularization, growth factors released from platelets; in particular, the transforming growth factor (TGF-β) and platelet-derived growth factor (PDGF) stimulate the proliferation of fibroblasts. Fibronectin and collagen production occur as the scar tissue becomes rich by fibroblasts. Fibroblasts synthesize collagen and prostaglandins (PG). Both act to create an unstructured connective tissue environment that allows new cells to migrate (Grazul-Bilska et al. 2003).

A number of PGs have been presented in the wound area, and their GAG side chains played a role in the stabilization and activation of growth factors. Sulfated PGs with chondrite sulfate (CS) and dermatan sulfate (DS) contribute to collagen polymerization. PGs provide a matrix for cellular attachment, and some PGs form triple complexes that moisten the tissue that promotes hyaluronic acid, cell survival to cover the wound site, and migration on granulation tissue (Brooks et al. 1994).

This stage takes place with a dynamic process occurring between the developing granulation tissue formation, fibroblasts, growth factors, and ECM. In this process, macrophages release growth factors that stimulate angiogenesis, collagen synthesis, and fibroblast proliferation after binding to the ECM. Endothelial cells bind ECM proteins through integrins, helping to advance the angiogenesis process (Arora et al. 1999).

3.4 Maturation and Restructuring

Remodeling is the stage characterized by the rearrangement of synthesized collagen up to a year after the initial wound formation. There is a balance between collagen production and destruction, characterized by the wound surface, it is the last stage of wound healing (Miller et al. 2003).

At this stage, with the transition of granulation tissue to a mature scar, a new epithelium is formed. This process is accompanied by high mechanical strength of the formed tissue, reduction of capillary amounts by mixing with larger blood vessels, decreasing cell density and metabolic activity of tissue, and lowering GAG content. Inflammatory cells gradually decrease. The early matrix skeleton consists of Type 3 collagen and fibronectin, while the final matrix skeleton is created

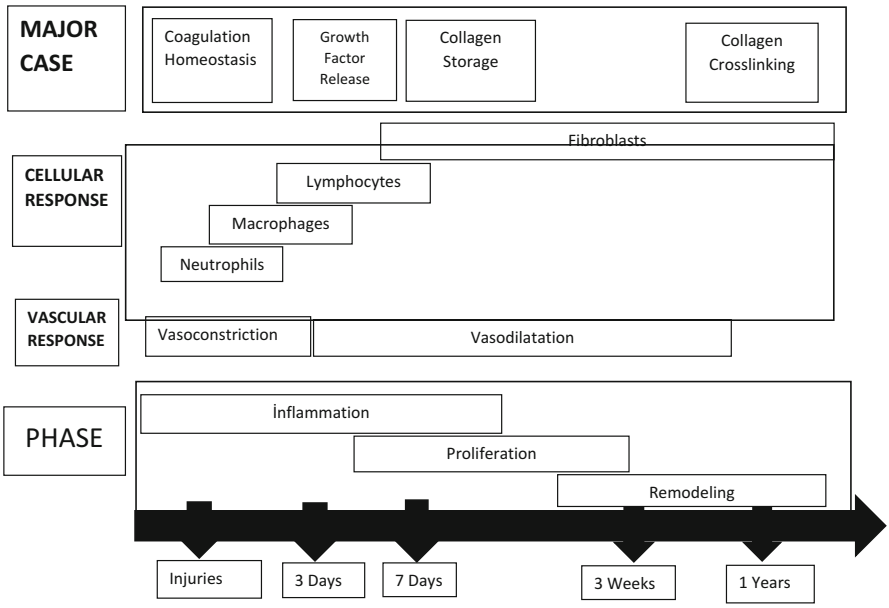

Fig. 1 Wound healing process Inflammation; the first response is the phase in which bacteria and foreign particles of neutrophils that come to the scar tissue within 48 h are inactivated by phagocytosis or toxic substances secreted. The phase in which proliferation angiogenesis, granulation tissue formation, collagen deposition, epithelization, and wound closure lasting approximately 2 days to 2 weeks, and a new matrix layer by fibroblasts, tissue in the wound area is restored. The remodeling phase creates a balance between collagen production and destruction, characterized by the wound surface, and wound healing takes place in three phases

by Type 1 collagen. The resistance of the scar tissue reaches 80% of the original tissue. The remodeling process continues for 21 days–2 years. In order to achieve optimum wound healing, good wound nutrition should be provided, pain should be reduced, clean wound and wound surface should be created, wounds should be protected from trauma and infection, systemic conditions should be corrected or improved, and expenses should be minimized (Ueno et al. 2001).

The mechanical strength of the formed tissue is equal to 25% in relation to the dermis and 80% with unchanged tissue after reconstruction for months (Ko et al. 2014). Considering that GAG activities can reduce inflammatory responses and ECM accumulation in the early stages of wound healing, an appropriate wound treatment at the start of injury of a GAG-rich material is expected to heal closer to normal skin (Mantle et al. 2001) (Fig. 1).

4 The Role of Extracellular Matrix in Wound Healing

Among the various molecules secreted by ECM, GAG has partners that have important roles in controlling all wound healing stages, acute wounds or severe wounds. These molecules participate in cell-cell and cell-matrix interactions, cell

proliferation and displacement, cytokine and growth factor signals. Thus, it modulates its biological activities locally. The ECM functions to guide the organized response characterized by hemostasis, inflammation, proliferation, and restructuring seen in wound healing. The effects of the various ECM components differ according to the wound stages. This dynamic and sequential order occurs as a result of the interaction of cell and growth factors (Ono et al. 1995).

ECM components are involved in every phase of wound healing. By interacting with cells and growth factors, they play a role in a dynamic shopping process that ultimately causes wound closure. More specifically, ECM components play a key role in stimulating cell proliferation and differentiation, directing cell migration, and modulating cellular responses. When the ECM loses its function (for example, in difficult to heal or chronic wounds), wound healing is slowed or stopped (Midwood et al. 2004).

5 Uses of Natural Polymers in Wound Healing

In addition to its presence in bacteria, hyaluronic acid, which is found in extracellular matrix and joint fluids in animal tissues, is important in terms of ensuring the elasticity of cartilage and tendons. However, it has played an important role in synovial fluid, in the vitreous of the eye, cell adhesion, cell mobility, embryo development, and in the living organism as a growth hormone promoter. It is also frequently used in wound healing, cancer metastases, and treatment of diseases such as nervous disorders and arthritis. Since hyaluronic acid polymers are organic substances that are capable of dissolving in water, they are also important because they act as viscosity increasing agents in the liquids they contain. Hyaluronic acid in the skin affects the passage rate of various substances through the skin. Hyaluronidase, which breaks down hyaluronic acid, facilitates the entry of various substances into the tissue. This enzyme, which is found in some microorganisms that cause pathogenic diseases, causes some pathogens to spread in the organism. It is known that the enzyme is also effective during fertilization (Wagener et al. 2017).

Heparin is the only GAG with anticoagulant properties. In other words, it inhibits the prothrombin-thrombin conversion and the effect of prothrombin on fibrinogen and prevents blood clotting. It is known to be used in the treatment of many diseases such as allergic rhinitis, asthma, and cancer. It is applied parenterally for the treatment of thrombosis, phlebitis, and embolism. Heparan sulfate oligosaccharide production is associated with the secondary accumulation of GM2 and GM3 ganglia in the brain, the formation of large cytoplasmic content in various brain cell types, in the accumulation of the C subunit of mitochondrial ATP synthesis, and the irregularity of GAP43 mRNA expression in brain tissues. It leads to very serious advanced mental retardation and premature deaths in the early onset of neurological diseases in children (Celebi and Onat 2006).

Chondroitin sulfate is involved in adults, part of learning and memory, and the neurohypophysis system in the hypothalamus. It also plays an important role in the damages and diseases in MSS. Chondroitin sulfate is the main stopper in the

component of glial wounds after damage in CNS. Surgical chondroitin sulfate regulates axonal regeneration and functional gains. It may also affect pathological stages in diseases such as epilepsy and Alzheimer's (Walker et al. 2005).

Dermatan sulfate is also found in the skin, blood vessels, heart valves, tendons, and lungs. It has a heparin-like antithrombic effect. But there are minimal whole blood anticoagulant and blood lipid cleansing activities. Dermatan sulfate is known to show therapeutic properties in coagulation, cardiovascular diseases, infection, recurrent wounds, and fibrosis (Kobayashi et al. 1997).

5.1 Drawbacks

While natural polymers used in wound healing provide advantages for patients, they also create disadvantages. Based on the wound type, healing time and effectiveness of the material used in patients are not the same. According to the researches, it is stated that the traditional cotton gauze allows moisture to evaporate from the wound surface, adheres to the wound bed and causes pain during removal, and therefore it is emphasized that the gauze dressing should be changed frequently. In order to overcome this problem, wound dressings produced with different features, especially modern dressings, meet the need in the medical field to a large extent. However, there are some problems with the dressings used in the treatment of wounds and burns. For example, the fluid amounts of different wound types should vary, and ideal dressings suitable for different wound types should be developed. Therefore, multidisciplinary studies are needed to further improve the existing dressings (Sidhu et al. 1998).

Natural polysaccharides have shown significant success in the treatment of chronic wounds for their ability to maintain anti-inflammatory and moist wound environment. However, in people with an allergic disease, natural polysaccharides cause excessive reaction and irritation due to the complex structure of the immune system. Therefore, control of the molecular weight of natural polysaccharides is expected to overcome this limitation. By selecting the desired molecular weight, the portion of the natural polysaccharide that can cause a hypersensitivity reaction should be simplified or removed. In addition, these properties in dry wound can also lead to inefficiency of wound healing process. By causing dehydration of the dry wound, it reduces blood flow and migration ability of epithelial cells around the wound area, thereby interrupting the formation of new tissue (Kumar et al. 1993).

Natural polysaccharides have been shown to be a great potential for medical, pharmaceutical, and biomedical applications, including wound dressings, biomaterials, and tissue regeneration, due to their economic, less toxic, and appropriate compatibility profiles. However, these polysaccharides have a lack of protein structures, very poor bio-stability, and difficulty in forming a "matrix" to bridge damaged tissue in the wound healing process, thus facilitating wound contraction and leading to scar formation (Yang et al. 2005). In the light of this information, the most reasonable product should be preferred in natural polysaccharide wound healing.

6 In Vivo and In Vitro Studies

From the polysaccharides, nanofibrillar structures, which are used in the treatment of wounds and burns, are obtained by the electrostatic spinning method. Among the homoglycans, cellulose, chitin, chitosan, dextran, alpha and beta glucan; among the heteroglycans, alginate, agar, agarose, carrageenan, pectin, gum and glycosaminoglycans (hyaluronic acid, heparin, chondroid sulfate, etc.) are polysaccharides frequently used in wound and burn dressings. Experimental animal wound models are still the most preferred models used to examine wound healing as they provide complex conditions that can best mimic wound formation and tissue repair. In these studies, the effectiveness of many natural polysaccharides has been evaluated. Alginate, chitosan, and hyaluronic acid from natural polysaccharides have been accepted as good candidates for the treatment of wounds for years. Its natural polysaccharide ability reduces scar formation in severe wound injuries due to its rich GAG content, which is known to support wound healing and leads to rapid granulation, vascularization, and reepithelialization, thereby providing absolutely minimal scar formation. Also, when the dressing is combined with the wound, an ion exchange reaction occurs between calcium in alginate and sodium in exudate, which produces a soluble gel that helps maintain a moist wound environment (Beer et al. 1997).

Regarding immune system activation, the release of pro-inflammatory cytokines such as IL-1β, IL-6, IL-8, IFN-γ, and TNF-α after wound injury also plays an important role in the wound healing process. Various important processes have been addressed with these cytokines, such as stimulation of keratinocyte and fibroblast proliferation in the wound site, synthesis and breakdown of ECM proteins, and regulation of the immune response. Expressions during the inflammatory phase of expressions have been shown to be intensely upregulated and strongly reduced after impairment of wound healing. Natural polysaccharide can stimulate human cells to produce cytokines with oligosaccharides (glu-glucan, xyloglucan, chitin, pectin, d-mannuronic, and l-guluronic). In particular, the β-glucan mechanism is mediated by many receptors, including dectin-1 receptor, Toll-like receptors complement receptor 3, scavenger receptor, and lactosylceramide. After binding to dectin-1 as the most important receptor, p-glucan stimulates the production of many cytokines or activates other immune and nonimmune reaction mechanisms (Iwamoto et al. 2005).

Chitosan is β-(1–4)-D glycosamino-N-acetyl-D-glycosamine, obtained by deacetylation of chitin. It has very low toxicity after biological degradation. Chitosan is widely used in the treatment of burns and traumatic wounds (33, 102). Chitosan and chitin accelerate wound healing by showing a chemoattractant feature for neutrophils in the early period of wound healing (Ishihara et al. 2006).

Ueno et al. (Ueno et al. 2001) and Ishihara et al. (Ishihara et al. 2006) found that chitosan increased the functions of polymorph nuclear neutrophils (PMNs) and macrophages in their studies. In addition to these features, it provides the proliferation and migration of vascular endothelial cells together with fibroblasts, and also prevents the secretion of interleukin-8 (IL-8) from fibroblasts (Ishihara et al. 2006).

Cross-linked chitosan containing fibroblast growth factor-2 (FGF-2) has been found to accelerate wound healing in diabetic mice. In the research conducted in second-degree burn injuries with chitosan gel containing epithelial growth factor

(EGF), it was emphasized that a faster epithelization was achieved compared to the control group. Chitosan stimulates fibroblast proliferation, the collagen needed, and natural hyaluronic acid synthesis from the wound edge (Kim et al. 2002).

Chitin and chitosan have been shown to stimulate canine PMNs in vitro to release leukotriene (LTB4), in vivo either directly or through complement activation, affecting canine PMNs by the production of arachidonic acid or cytokine. Glucuronic acid N-acetylglycosamine is one of the most hygroscopic molecules in nature, with a disaccharide structure. This hygroscopic feature of hyaluronan helps weaken the bond between the ECM and the cells, thereby dividing the cells by migration. Due to its high viscous feature, it prevents the contact of viral and bacterial passages into cells by creating pericellular region rich in hyaluronan. Hyaluronan also has antioxidant action as a free radical scavenger. Hyaluronan and derivatives have been used for wound healing, and it has been reported that both hyaluronan and derivatives show a bacteriostatic effect and protect the wound area against microorganisms (Presti and Scott 1994).

Martins et al. in their study, they found that a polysaccharide-rich fraction of *Agaricus brasiliensis* can regulate host response by activating both pro- and anti-inflammatory mechanisms, thereby increasing TNF-a and IL-1β production by human monocytes through modulation of Toll-like receptor 4 (Presti and Scott 1994). In addition, even after TLR blockade, these polysaccharides still activate monocytes to produce sufficient levels of IFN-γ, IL-1, and IL-10. TNF-a and IFN-γ are considered important agents of antimycobacterial cytokine cascade, and IL-10 is considered an inhibitory cytokine that is important for adequate balance between inflammatory and immunopathological responses. On the other hand, IL-1β is known as a critical inflammatory agent that plays a role in neutrophil mobilization, endothelial cellular adhesion, and white blood cell infiltration (Dinarello 1996).

Zhao et al. in their study, they determined the wound healing effect and mechanism of Astragalus membranaceus polysaccharide treatment through in vitro and in vivo studies. The results showed that this polysaccharide can promote fibroblast spread in the human skin and accelerate the progression of the cell cycle, and also significantly confirms the secretion of TGF-β1, bFGF, and EGF, which substantially confirms accelerated wound closure in the mouse wound. TGF-β1 is an important promoter in fibroblast proliferation and ECM secretion, and while preventing its deterioration, EGF and bFGF are important stimulants in the formation of reepithelialization and keratinocyte migration in wound healing. In addition, pain and mechanism of pain signals, including peripheral and central processing, are related to the modulation of keloids and TGF-mod involved in hypertrophic pathogenesis (Zhu et al. 2012).

7 Conclusion

It is very important for the materials used in wound healing and wound management. Natural polysaccharides used in the treatment of chronic or acute wounds provide many advantages in terms of wound repair and time. Natural polysaccharides are

more preferred than difficult and laborious methods with their biocompatible, biodegradable, nontoxic, antitumor, antimicrobial, and wound healing properties.

With the developing technology, the use of natural polysaccharide should be increased in wound healing and the product with this feature should be developed. Studies have increased the variety of products that protect the wound surface and speed up the healing and reveal new treatment options. However, more studies are needed to create effective evidence.

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