Chapter 6

The Strategies of Natural Polysaccharide in Wound Healing

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Additional information is available at the end of the chapter

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Abstract

Severe or chronic wounds related to diseases or serious incidents have received big attention from not only a scientific standpoint but also a business perspective. Therefore, an effective treatment to abridge the long-term hospitalization of severe wound becomes indispensable. Glycosaminoglycan (GAG), one of the extracellular matrix molecules produced by fibroblasts, participates in cell-cell and cell-matrix interactions, in cell proliferation and migration, and in cytokine and growth factor signaling associated with all phases of wound recovery. Natural polysaccharide, for example, calcium alginate, which consists of mainly differing ratios of D-mannuronic and L-guluronic acid and rich of calcium ions, has been demonstrated to functionalize the glycosaminoglycan activity during wound healing. Once the trigger of the underlying wound healing mechanisms was understood, it should be possible to find ways to enhance and resolve the wound healing process in the patient with conditions and may lead to the potential for treatment alternatives in the future clinical field.

Keywords: glycosaminoglycan (GAG), extracellular matrix (ECM) molecules, cytokines, natural polysaccharide, wound healing

1. Introduction

Wound injuries are the most common health problem people faced in decades and continuously demand advanced wound management strategies to obtain optimal healing. Wound injuries can range from small wounds caused by daily activities to chronic or severe wounds caused by diseases or serious incidents. Besides the type of wound being treated, the effectiveness of wound management also involves a better understanding of different factors such as

the healing process and the physical-chemical properties of the available dressings [1]. Mostly in small wounds, tissue injuries will heal completely with normal healing phases within weeks [2]. On the other hand, severe or chronic wounds are hard to heal within months or a year and often reoccur with persistent inflammation [3], which represent major challenges to patients medically and financially. Therefore, proper wound dressings that have the ability to accelerate the wound healing phases and reduce the healing time simultaneously are required to overcome this problem.

Glycosaminoglycans (GAG) are extracellular matrix molecules that have significant roles in the control of the all wound healing phases, either an acute wound or severe wound, such as an effective mediator of angiogenesis and inflammation [4, 5] and promote the wound recovery by leading to rapid granulation, vascularization, and reepithelialization [6]. As a GAG-rich content, the use of natural polysaccharide as wound dressing-based material is proposed to enhance the healing phases, especially to abridge the long-term healing mechanism in severe wound injury, determined by several studies and clinical trials. This chapter will further discuss the detail mechanisms and efficacy of natural polysaccharide in accelerating the wound healing process, thereby intended to encourage the advanced strategies for future wound management.

2. The involvement of glycosaminoglycan during wound healing

Generally, wound healing has been represented with the complexity and overlapping of its phases. These processes integrate a dynamic interaction between cells and extracellular matrix (ECM) that trigger tissue or organ regeneration. The significant roles of ECM and its components during each stage of the healing process are represented by structural matrix provision and function of signal transduction compliance in the dynamic of biological reactions during each stage of the healing process [7, 8–11].

ECM provides structural and functional integrity to connective tissues and organs [12]; yet its synthesis and deposition mainly occur in response to growth factors, cytokines, and mechanical signals mediated via cell surface receptors [13]. In the case of wound healing, ECM consists of at least four major classes [8]: (1) structural proteins such as the collagens and elastin; (2) multidomain adhesive glycoproteins such as fibronectin, vitronectin, and laminin; (3) glycosaminoglycan (GAG) such as hyaluronic acid (HA), proteoglycans (PGs) including versican, syndecans, glypicans, aggrecan, and perlecan (chondroitin sulfate (CS)/dermatan sulfate (DS), and heparin sulfate (HS))—and keratin sulfate (KS), often in large amounts; and (4) matricellular proteins such as secreted protein acidic and rich in cysteine (SPARC, also known as osteonectin and BM-40), thrombospondins 1 and 2, tenascin C and X, and osteopontin.

GAG is the important constituent of the extracellular matrix found on cell surfaces [14] and widely distributed in connective tissues. GAG is composed of characteristic repeating disaccharides, with specific monosaccharides sulfated at each of C_2, C_3, C_4, and C_6 [15]. These compounds are highly anionic polymers, which interact with many cationic species (such as ions and proteins) due to the presence of the carboxylic acid and sulfate functional groups [16]. The negative ion charge of GAG molecules carries was considered substantial in many biological processes.

Among the various molecules secreted by ECM, the GAG has partners that have significant roles in the control of the all wound healing phases, either acute wound or severe wound. Those molecules participate in cell-cell and cell-matrix interactions, in cell proliferation and migration, and in cytokine and growth factor signaling, thus locally modulating their biologic activities. In an acute wound, the healing progresses through the normal phases of wound healing (hemostasis, inflammation, proliferation, and remodeling) within weeks, while in a severe wound, those phases do not progress normally, mostly within months or years, thus needing an appropriate supplementary treatment to enhance the healing process. Therefore, GAG or GAG-containing material treatment is expected to become a key to turning and accelerating not only acute wound but also especially severe or chronic wound healing problems.

2.1. Role of glycosaminoglycan in acute wound healing

2.1.1. Hemostasis phase of wound healing

Hemostasis is the beginning of the wound healing process and may be defined as the interaction between platelets and vessel of vascular injury. The vital mediators of hemostasis are fibrin, platelets, and blood vessels. In the first 1 or 2 hours after injury, wound repair starts with the formation of a fibrin matrix through the proteolytic cleavage of fibrinogen by thrombin, and fibrin directly binds to platelets to produce a clot [17–21]. The α granules of platelets release numerous growth factors, such as PDGF, TGF-α, TGF-β, bFGF, IGF-1, and VEGF [22, 23]. Further, PDGF and IGF-1 call up and activate the fibroblasts as well as synthesize GAG and collagen to lead the migration and proliferation of the cells into the wound site [24, 25].

The enzymes of the fibrinolytic resist the clot formation. On the other hand, serpins ensure that excessive fibrinolytic activity does not occur. The ECM contains a network of scaffold proteins that are linked by GAG. GAG, especially HS, plays a key role as anticoagulants that have important acts to manage the regulations of many of the serpins [15]. HS represents 50–90% of the total GAG content [25] and is only in contact with blood when an injury occurs [26, 27]. HS has been identified binding with more than 100 proteins involved in hemostasis, many growth factors, proteins involved in lipid metabolism, and proteins of the ECM [28]. In addition, HS maintains hemostasis as an effective mediator of angiogenesis at the surface of endothelial cells [4].

Summarily, the hemostasis phase begins the healing process, generates blood clot formation which maintains the structure of vessels, and provides a temporary matrix, secreting cytokines and other growth factors, in order to prepare the wound bed for the next phase of the healing process.

2.1.2. Inflammatory phase of wound healing

The inflammatory response is elicited by infection or tissue injury involved in the distribution of blood components (plasma and leukocytes) to the damage site [29, 30]. This phase occurs in the next 24–48 hours after injury on average accompanied by inflammation symptoms, such as redness, body heat, swelling, and pain around the wounded place [31]. Once the bleeding is controlled, the key cells of the inflammatory response such as neutrophils, macrophages, and

lymphocytes assemble into the wound site, simultaneously release a large number of active mediators (cytokines and growth factors), and thus stimulate the inflammatory phase [32–34].

HA, a non-sulfated GAG of the ECM, is involved in a significant process of the inflammatory phase. During this phase, HA assembles in the wound bed and regulates early inflammation to modulate inflammatory cell and fibroblast cell migration, pro-inflammatory cytokine synthesis, and the phagocytosis of invading microbes [5]. Moreover, HA may bind and improve the efficiency of chemokines to neutrophils. Butler et al. revealed that HA appeared able to present stimuli to neutrophils [35]. HA on the endothelial surface was increased as well as the efficiency of recruitment of neutrophils. In the inflammatory phase, neutrophils collagenase and elastase eliminate damaged tissue from the temporary matrix of the wound site, while monocytes transform into macrophages and phagocytose fragments of denatured ECM debriding the wound site and inactivating any source of microbial infection through the activity of secreted proteases.

At sites of inflammation, the low-molecular-weight HA fragments (accumulated from degradation of high-molecular-weight HA) can initiate Toll-like receptor 2 and Toll-like receptor 4 induction of pro-inflammatory cytokines IL-6, TNF-α, and IL-1β [36]. Furthermore, the growth factors and cytokines released by the inflammatory cells induce the migration and proliferation of fibroblast and keratinocyte, which synthesize the levels of HA. All along the reepithelialization process, where epithelial cells migrate across the new tissue to form a barrier between the wound and the environment, the level of HA was found significantly elevated [37]. The secretion of cytokines such as TGF-β, PDGF, FGF-2, IL-1, and TNF-α modulate collagen deposition by fibroblasts and penetration of new blood vessels into the wound site.

2.1.3. Proliferation phase of wound healing

During the proliferation phase of wound healing, over the next 2 or 3 days and lasts for about 2 weeks thereafter, the layer of a new matrix by fibroblasts restores the tissue at the wound site. The other mesenchymal cells also enter the inflammatory site of the wound in response to growth factors that are necessary for the stimulation of cell proliferation [38]. Moreover, fibroblasts, endothelial cells, and keratinocytes produce IGF-I, FGF-2, TGF-β, PDGF, and VEGF, promoting cell migration and proliferation, matrix synthesis, and angiogenesis.

The fibroblasts synthesize collagen and PGs. Both of them act to form an unstructured connective tissue medium that provides new cells to migrate. A number of PGs were presented in the wound site, and their GAG side chains were involved in the stabilization and activation of growth factors [15]. Sulfated PGs with CS and DS contribute in collagen polymerization [39], and HS PGs on cells can create anchors to the surrounding matrix [40]. The PGs provide a matrix for cellular attachment, and some PGs (hyalectans) form ternary complexes with HA hydrating the tissue promoting cell survival and migration above the granulation tissue to cover the wound site.

2.1.4. Remodeling phase of wound healing

Remodeling is the final phase of wound healing which is achieved over longer periods of up to a year after the initial wound injury [41]. This phase is characterized by wound surface

contraction [42]. During this phase, new epithelium forms along with the transition of granulation tissue to a mature scar. This process is accompanied by high mechanic strength of the formed tissue, reduction of capillary amounts by combining into bigger blood vessels, lowering cell density and metabolic activity of the tissue, and lowering the content of GAG [43, 44]. The mechanic strength of the formed tissue equals 25% related to the dermis and equals 80% related to the unchanged tissue after many months of reconstruction [43, 45, 46]. Considering that GAG activities are able to reduce the inflammatory responses and ECM deposition in the early phases of wound healing, a proper wound handling in the beginning of injury with a GAG-rich-containing material is expected to heal the wound more closely to normal skin and reduce the period of this phase.

2.2. Role of glycosaminoglycan in severe or chronic wound healing

Severe or chronic wounds, hard-to-heal wounds related to diseases or serious incidents, were also commonly encountered instead of acute wounds. Mostly, this issue has been associated with the aging of the population. Unlike acute wounds, the treatment and management of severe wounds represent major challenges to patients medically and financially, resulting in a long-term recovery.

Severe wounds are frequently characterized by persistent injury and prolonged inflammation, high incidence of bacterial biofilms, and excessive proteolysis [3]. Impairment of macrophage function and angiogenic response is also suggested, which are mostly related to severe wounds healing process [47, 48]. Due to the prolonged inflammation, an excessive recruitment of inflammatory cells to the wound bed will be incurred and produced by the large numbers of neutrophils. It is known that neutrophils can eliminate damaged tissue from the temporary matrix of the wound site and prevent microbial infection. On the other hand, however, the unmanageable neutrophils' potential to kill pathogens also can lead to excessive protease production that initiates significant tissue damage to the host which is harmful to wound healing as they cause degradation of the ECM and growth factors [16]. Furthermore, the inefficient cell proliferation due to ECM molecule degradation within the wound leads to impaired angiogenesis that indicates further wound bed defacement and impaired healing. Hence, in order to conquer this issue, the prevention of prolonged inflammation is a goal strategy in severe wound therapy.

GAG has been found to bind to neutrophils, macrophages, and lymphocytes which are the key cells of the inflammatory response. The effect of excessive protease production caused by too many activated neutrophils in the wound site can be inhibited by electrostatic binding with certain anionic polymers such as GAG or functionalized dextrans [16]. The highly anionic nature of GAG that was expected would be ion pairing with the cationic neutrophils to interfere the activity of these cationic proteins via charge interactions. Therefore, it may be possible that by this mechanism the excessive neutrophil recruitment is reduced and the wound can pass from the inflammatory stage to the next stage of healing.

However, after serious tissue injury, the glycanases and proteases can destroy GAG [49]. The lack of GAG in severe wounds can be fixed with the addition of GAG-containing material, such as a natural polysaccharide, directly into the wound site as a wound dressing. With the

rich source of GAG at the wound environment and a better understanding of GAG roles in healing processes, it has been possible to formulate therapeutic strategies which are expected to accelerate severe wound healing.

3. Natural polysaccharide in wound healing

3.1. The properties of natural polysaccharide

Glycosaminoglycan (GAG) has been shown to perform significant roles in cell signaling and development, angiogenesis, anticoagulation, and co-receptors for growth factors, which belong to the control of the all wound healing phases, both of acute wound and severe wound. GAG is an enormous complex of carbohydrate molecules that interact with several proteins involved in physiological and pathological processes [50, 51]. GAG, with a molecular weight roughly around 10–100 kDa, is a linear negatively charged polysaccharide. This electrostatic characteristic is useful for managing the excessive protease production through ion pairing with the cationic neutrophils and interfering the activity of these cationic proteins via charge interactions [16]. Once the excessive neutrophil recruitment is reduced as well as the excessive protease production, the wound can pass efficiently from the inflammatory stage to the next stage of healing, especially for severe wound injury.

Polysaccharides, especially natural polysaccharide, have been extensively used in wound-dressing development due to their properties such as being biocompatible, nonimmunogenic, and antimicrobial [52–54]. They appeared as abundant sources in many different forms of plants and production in the body. Based on their availability, a natural polysaccharide with different chemical structures and physical properties represents a large source of materials for progressive applications in the future, especially in the domain of biomaterials for the medical field [55, 56].

Containing a beta-1,3-D-glucan linker, natural polysaccharides contribute to the wound healing process because of their ability to stimulate immune system activation by activating macrophages that clean up the wound site after injury [57]. The macrophage is one of the major inflammatory cells in wounds. It has many substantial functions during wound healing, such as host defense, the promotion and resolution of inflammation, the removal of apoptotic cells, and the support of cell proliferation and tissue restoration following injury [58]. In several studies, natural polysaccharides have been shown to enhance macrophage cytotoxic activity against tumor cells and microorganisms and activate phagocytic activity by escalated reactive oxygen species (ROS) and nitric oxide (NO) production [59–61]. These abilities are useful for enhancing the quality of the wound healing process.

3.2. The effect of natural polysaccharide structure in wound healing: animal studies

Alginate, chitosan, and hyaluronic acid are mainly natural polysaccharides that are considered as good candidates for the management of wounds in decades. Alginate, as a prominent example of a natural polysaccharide with the abundance of GAG, has been utilized to become platforms used for fabricating wound dressing materials. Spun as calcium alginate wound

dressing, a severe wound injury in swine model treated with this material exhibited a rapid reepithelialization and less scar formation, which appeared with a smooth wound, compared to commonly used wound dressing [62]. The ability of natural polysaccharide reduces scar formation in severe wound injury due to its rich content of GAG which was known to promote wound healing and leads to rapid granulation, vascularization, and reepithelialization, thus yielding a minimum scar formation certainly [6]. As well, once the dressing is attached with the wound, an ion exchange reaction occurs between the calcium in the alginate and the sodium in the exudate, producing a soluble gel which in turn helps maintain a moist wound environment [63]. A moist wound environment will prevent the scab's formation and facilitate the growth and migration of cells to optimize the formation of new tissues.

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 takes an important part during the wound healing process. Various crucial processes at the wound site, such as stimulation of keratinocyte and fibroblast proliferation, synthesis and breakdown of ECM proteins, and regulation of immune response, were handled with these cytokines [64]. Their expressions were shown to be intensely upregulated during the inflammatory phase of healing and strongly reduced after wound healing was impaired [65].

Natural polysaccharide, by its oligosaccharides (β-glucan, xyloglucan, chitin, pectin, D-mannuronic, and L-guluronic), can stimulate human cells to produce cytokines [66, 67]. Particularly, the mechanism of β-glucan is mediated by several receptors including dectin-1 receptor, Toll-like receptors (2, 4, 6), complement receptor 3, scavenger receptor, and lactosylceramide [68]. Once binding to the dectin-1, as the most important receptor, β-glucan stimulates the production of many cytokines or activates other immune and nonimmune reaction mechanisms [69]. Martins et al. demonstrated that a polysaccharide-rich fraction of *Agaricus brasiliensis* is able to regulate the host response by activating both pro- and anti-inflammatory mechanisms, thus increasing the production of TNF-α and IL-1β by human monocytes through modulation of Toll-like receptor 4 and Toll-like receptor 2 expression [70]. In addition, even after TLR blockade, these polysaccharides still activated the monocytes to produce considerable levels of IFN-γ, IL-1β, and IL-10. TNF-α and IFN-γ were recognized as the important agents of the anti-mycobacterial cytokine cascade, and IL-10 was considered as an inhibitory cytokine which is important to the adequate balance between inflammatory and immunopathological responses [71]. On the other hand, IL-1β is known as a critical mediator of inflammation which is involved in neutrophil mobilization, cellular adhesion to the endothelium, and white blood cell infiltration [72, 73]. Zhao et al. determined the wound healing effect of an *Astragalus membranaceus* polysaccharide treatment and its mechanism through in vitro and in vivo studies. The results showed that this polysaccharide was able to promote human skin fibroblast propagation and accelerate cell cycle progression, as well as the reepithelialization, revascularization, and cytokine secretion of TGF-β1, bFGF, and EGF which significantly confirmed the accelerated wound closure in mouse wound model [74]. TGF-β1 is an important promoter in the fibroblast proliferation and the secretion of ECM and inhibits its degradation, while EGF and bFGF are important stimulators in the formation of reepithelialization and keratinocyte migration in wound healing [75]. Moreover, the pain and the mechanism of pain signals including peripheral and central processing are also related to the modulation of TGF-β, which is implicated in the pathogenesis of keloids and hypertrophic

scarring [76]. The use of calcium alginate dressing for severe wound injury treatment in the animal model demonstrated high levels of TGF-β1, TGF-β2, and TGF-β3, suggesting that it might contribute to reduced pain perception [62].

3.3. The application of natural polysaccharide in clinical trials

There are also several clinical trials of natural polysaccharide for wound repair. A natural polysaccharide that contains rich GAG has been widely used in the medical field as electrospun regenerative materials in the act of matrices that mimic tissues which are being replaced during wound healing. HA-based dressings have been used for chronic wound ulcer treatment of various etiologies, burns, and epithelial surgical wounds. The results revealed that HA significantly upgraded the healing process of wounds compared to traditional standards of care [77]. In line with this result, chitosan and alginate, fabricated as gelling fiber dressing, have been examined to accelerate the healing of patients with chronic non-healing wounds [78, 79]. This dressing has the ability to gel when in contact with wound fluid, less painful to remove, suitable for moderate to high exudate, reduced bioburden, and maintain hemostasis. Taken together, all of these benefits nominate natural polysaccharide as an advisable material in accelerating the wound healing process.

4. Limitation to using natural polysaccharide in wound healing

Generally, natural polysaccharide has demonstrated considerable merit as a treatment for chronic wounds for their anti-inflammatory and moist wound environment preservation abilities. Despite, especially for atopy people (the people with genetic tendency to develop allergic diseases), a natural polysaccharide may induce the immune system to overreact and cause irritation due to its heterogeneous complex structure. Hence, the control of the molecular weight of natural polysaccharide is expected to overcome this limitation. Through the selection of desirable molecular weight, we could simplify or remove the excessive part of natural polysaccharide that may cause the hypersensitivity reaction. Additionally, in the dry wound, these properties may also lead to the inefficiency of the wound healing process. They may cause dehydration to the dry wound, thus reducing blood flow and the epithelial cells' migration ability around the wound site which interrupts the creation of new tissue. As evidence, reepithelialization of the wound site is more rapid under moist conditions than under dry ones with natural polysaccharide wound dressing treatment [80–82]. With controllable molecular weight, it is probable that the potential of natural polysaccharide in accelerating the wound healing process can be utilized as well into several types of wound injury and patient background.

A natural polysaccharide is the element of human dermal ECM [83–86]. As naturally occurring compounds, they have been demonstrated as a great potential for medical, pharmaceutical, and biomedical applications, including wound dressings, biomaterials, and tissue regeneration, due to their economical, less toxic, and favorable compatibility profile. However, possessing a lack of protein structure, natural polysaccharide exhibits a very poor bio-stability and difficulty to assemble a "matrix" to bridge the damaged tissue during wound healing process, therefore facilitating wound contraction and leading to scar formation [87–89]. To address this limitation, natural polysaccharide has been combined with the other natural polymers or synthetic polymers to yield the desired bioactive material.

5. Conclusion and future perspective

Wound dressings have a significant function in the management of wound recovery and have been continuously developed upon to improve the quality of the healing process. In this respect, a natural polysaccharide, with GAG-rich content, has been shown as a potential candidate to enhance the healing process of the wound, especially a severe wound, due to its outstanding properties. The detailed mechanism of natural polysaccharide involvement in wound healing was presented in this chapter, and it is expected to raise further wound management strategies. For example, recently, 3D bioprinting was expanded in tissue engineering for personalized regenerative medicine; hence, natural polysaccharide can be considerably utilized as the bio-ink for the printing of various types of structures as scaffolds as the desired function. The combined therapeutic potential of natural polysaccharide and proper development technique would be a promising potential not only in the wound management field but also the other medical applications.

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