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Review Article

Chitosan as a starting material for wound healing applications

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ABSTRACT

Chitosan and its derivatives have attracted great attention due to their properties beneficial for application to wound healing. The main focus of the present review is to summarize studies involving chitosan and its derivatives, especially N,N,N-trimethyl-chitosan (TMC), N,O-carboxymethyl-chitosan (CMC) and O-carboxymethyl-N,N,N-trimethyl-chitosan (CMTMC), used to accelerate wound healing. Moreover, formulation strategies for chitosan and its derivatives, as well as their *in vitro*, *in vivo* and clinical applications in wound healing are described.

1. Introduction

Wound healing is a spontaneous process, which might be impaired in large or difficult-to-heal, chronic wounds. In such cases, and when autologous skin grafts are not available, biopolymers may be used to promote and initiate the normal dermal and epidermal wound healing process [1]. Dermal wound healing is a complex biological process, which includes four overlapping steps. These are the inflammatory phase immediately after the lesion has occurred, the migratory, proliferative and maturation phase resulting in remodeling [2]. Among other factors important in wound healing, the extracellular matrix (ECM) has a key role in orchestrating and guiding cell phenotype, adhesion, migration and proliferation. The ECM comprises proteins synthesized by fibroblasts, including proteoglycans (e.g., chondroitin sulfate), keratin sulfate, heparin sulfate and fibrous proteins like laminin, type IV collagen and elastin. In addition, the ECM serves as a deposit for growth factors, proteases, cytokines and chemokines [3]. ECM proteins have an important role during the proliferative phase by providing the mechanical support necessary for angiogenesis in the newly forming granulation tissue [4]. Beside the formation of granulation tissue in the dermis, wound re-epithelialization is another important process during which keratinocytes and fibroblasts migrate into the wound area [5].

Skin wounds are the result of disruption of normal tissue anatomy and may be classified by the type of repair process involved as acute or chronic [6]. Acute wounds originate from superficial scratches to deep injuries and heal completely with minimal or no scar formation within a timeframe of 3 weeks. Chronic wounds, such as certain types of ulcers or diabetic wounds [2] start to develop when the acute wound fails to heal after a minimum time period of 3 months. Based on the etiology, type, depth and clinical appearance, wounds are classified as shown in Fig. 1 [6,7].

Abbreviations: CMC, carboxymethyl-chitosan; CMTMC, O-carboxymethyl-N,N,N-trimethylchitosan; EGF, epidermal growth factor; FGF, fibroblast growth factor; HMWC, high molecular weight chitosan; LMWC, low molecular weight chitosan; PDGF, platelet-derived growth factor; TMC, N,N,N-trimethylchitosan.

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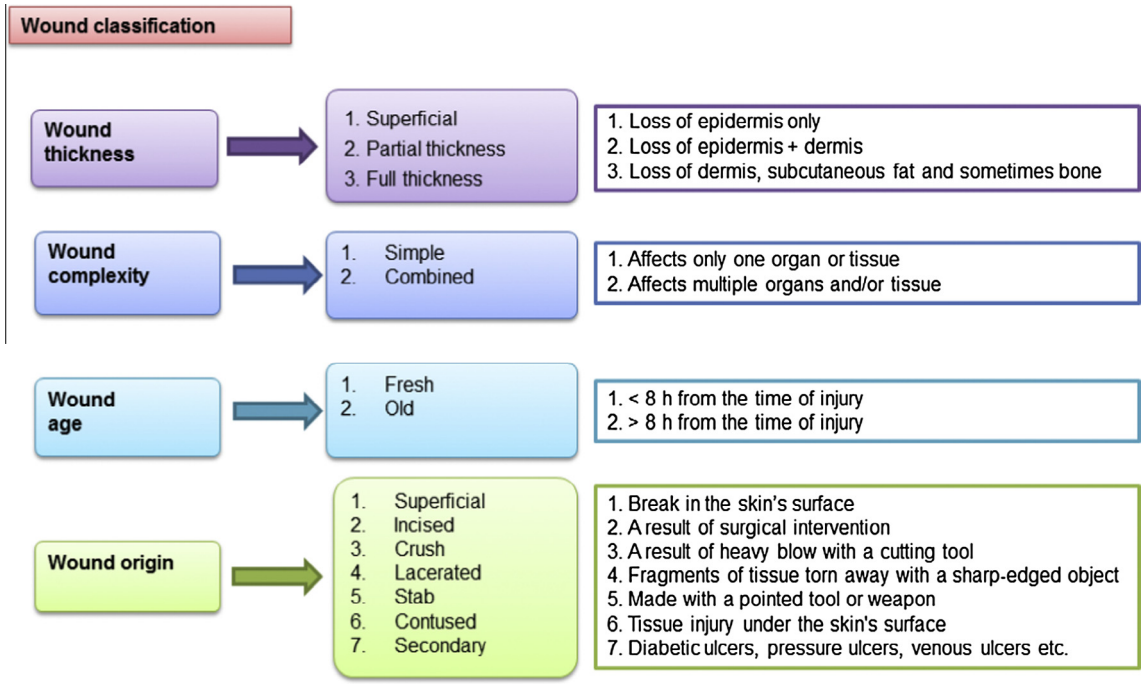

Fig. 1. Wound classification with respect to thickness, complexity, age and origin.

The treatment of chronic or bacteria-infected wounds necessitates new technologies. In this view, the use of biocompatible, absorbable polymers such as pectin [8], chitin and its derivative chitosan [9], gelatin [10], polycaprolactone [11], hyaluronic acid [12], and others have been described.

Having a unique set of biological properties including biocompatibility, biodegradability and low to absent toxicity [13], chitosan has been found to be an attractive material for wound healing applications. In addition, chitosan has antibacterial, hemostatic and mucoadhesive properties [14], and may act as a wound healing accelerator [15].

Chemically, chitosan is a linear aminopolysaccharide, composed of glucosamine and N-acetyl glucosamine units linked by β (1–4) glycosidic bonds formed by N-deacetylation of chitin (its parent polymer). Chitin, the second most abundant biopolymer, is mainly found in exoskeletons of crustaceans and cell walls of fungi [16]. Chitosan is a polycationic polymer featuring free acetamide groups and hydroxyl functions linked to the glucopyranose rings that are susceptible to react through nucleophilic attack [17]. A wide range of chitosan functionalizations can thus be performed through selective modification of the free amino groups [18,19]. According to the Chemical Carcinogenesis Research Information System [20], chitosan has no mutagenic effects, which makes it a candidate for biomedical application.

Chitosan has been used in gels, micro- or nanoparticles, and films. Its physical and biochemical properties can be further tailored to meet conditions in wound healing applications [18,21–24]. Beside numerous chitosan derivatives, trimethyl chitosan (TMC), N,N-carboxymethyl chitosan (CMC) and O-carboxymethyl-N,N,N-trimethyl chitosan (CMTMC) have been synthesized, raising increasing interest due to their enhanced solubility, antibacterial activity, ability to complex drugs or DNA [25], and biocompatibility [26–28]. The mechanism of action in the promotion of wound healing by chitosan derivatives is still debated but is suggested to depend on the type of functionalization.

This review describes the uses of chitosan as a starting material and dressing for wound healing applications, with a specific focus on clinical studies performed to date. Specific derivatives based on trimethylated and carboxymethylated chitosan will be described as well. We focused on chitosan derivatives, which are known to promote wound healing. Other chitosan derivatives developed for other purposes have also been described in literature in the context of wound healing [21,29–31]. Specifically, for diabetic wounds, where healing fails, other chitosan derivatives have been proposed [32] to increase fibroblast migration and collagen deposition in diabetic mice [33].

2. Chitosan as wound healing promoter

2.1. Chitosan history

Chitosan, a chitin derivative, was discovered in mushrooms in 1811 by the French chemist Henri Braconnot and named in 1859 by C. Roget [34]. Following D-glucosamine synthesis in 1903 by Fischer and Leuchs, Karrer decomposed chitin with chitinase in the year 1929. The absolute configuration of glucosamine was determined by Haworth in 1939 [35]. Only in 1970, Prudded's research concluded that glucosamine and N-acetyl-D-glucosamine accelerated wound healing processes [36]. Chitosan and its derivatives were first used for skin and wound healing in the 1980s. A chitin material, Beschitin®, was used for human application as dressing for skin and nasal wounds [37]. From then on chitin and chitosan were consistently shown to enhance wound healing in animals [38] and human subjects [39].

2.2. Chitosan toxicity

2.2.1. In vitro toxicity of chitosan

In vitro toxicity of chitosan is closely related to both its degree of deacetylation (DD) and to its molecular weight (MW) [40]. The effect of two types of micron-sized chitosan particles with different molecular weight – low (LMWC; 50–190 kDa) and high (HMWC; 310–375 kDa) molecular weight chitosan – was studied on a keratinocyte cell line (HaCaT) using the MTT assay. Both chitosan species promoted proliferation of HaCaT cells, with HMWC inducing a