International Journal of Biological Macromolecules 89 (2016) 725–736

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

# Wound dressing based on chitosan/hyaluronan/nonwoven fabrics: Preparation, characterization and medical applications

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ARTICLE INFO

*Article history:*
Received 26 March 2016
Received in revised form 28 April 2016
Accepted 30 April 2016
Available online 2 May 2016

*Keywords:*
Hyaluronan
Chitosan
Nonwoven fabrics
Cytotoxicity
Healing properties

ABSTRACT

Thin layers of chitosan (positively charged)/sodium hyaluronate (negatively charged)/nonwoven fabrics were constructed by polyelectrolyte multilayer pad-dry-cure technique. Pure chitosan (CS) was isolated from shrimp shell and immobilized onto nonwoven fabrics (NWFs) using citric acid (CTA) as cross linker and solvent agents through a pad-dry-cure method. The prepared thin layer of chitosan citrate/nonwoven fabrics (CSCTA/NWFs) were consequently impregnated with hyaluronan (CSCTA/HA/NWFs) in the second path through a pad-dry-cure method. Chitosan/hyaluronan/nonwoven fabrics wound dressing was characterized by different techniques such as FTIR-ATR, TGA and SEM. The antibacterial activity and the cytotoxicity of the dressing sheets were evaluated against *Escherichia coli* (*E. coli*) and *Streptococcus aureus* (*S. aureus*), mouse fibroblast (*NIH-3T3*) and keratinocytes (*HaCaT*) cell lines, respectively. The cell-fabrics interaction was also investigated using fluorescence microscope, based on live/dead staining assay of *3T3* cells. The healing properties of the new wound dressing were evaluated and compared with the control sample.



## 1. Introduction

Healing of wound is a complicated process of restoring cellular structures and epidermal tissue in damaged part to its normal condition as closely as possible [1]. This dynamic process consisting of three continuous, overlapping phases, the first phase is the inflammation phase which occurs immediately after wounding and continue for 24–48 h, hemostasis is the beginning of this phase which leads to the inflammation. Platelet-derived growth factors are released into the wound that promote the chemotaxis and proliferation of neutrophils in order to clean the wounds from bacteria, dead tissues or debris [2–6]. The second phase is fibroblast proliferation; in this phase, collagen is produced in order to supply the structure to wounds and replaces the fibronectin–fibrin matrix. The last phase is the remodeling which approximately starts after two or three weeks and can take more than two years. In this phase, new collagen forms and wound strength gradually increases. Many factors affecting on healing process as the type of damage, the ability of the tissue to repair and the general state of the host's health [7–9]. Layer-by-layer (LbL) assembly is a technique that was used in large scale due to its extraordinary advantages in the preparation of multilayer films [10–14]. Multilayer films were formed as the result of impregnation procedure, different alternating polyelectrolyte layers of oppositely charged was successfully assembled on flat surfaces. Also, these films were easy synthesized on different substrates. On other hand, these layers had varies features as uniform,

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http://dx.doi.org/10.1016/j.ijbiomac.2016.04.087
0141-8130/

continuous, easily tailored, and resistant to protein adsorption in especial cases [15–27].

Chitosan is a cationic biopolymer, owing to its characteristic as nontoxicity, biodegradability, biocompatibility, antimicrobial activity and improving the healing of wounds; chitosan has been widely used as topical dressing in wound management [28–35]. Chitosan is characterized by three functional groups, an amino, acetamido and hydroxyl groups. Cationic character of amino group (after its potentization) gives good opportunities for it to react with other compounds, and the key properties of chitosan includes that it is bactericidal, fungicidal, and has immune-enhancing properties [28,36,37]. Also, chitosan is water soluble in acidic media due to the protonation of amino group on the second carbon of glucosamine [37]. Recently, chitosan has received extensive attention because it is a promising natural substances used in the biomedical, food, and chemical industries [36]. Chitosan and its derivatives have been shown an excellent antimicrobial activities towards most of living microorganisms as yeast, fungi and bacteria, this make many scientists be attention by this biopolymers [38,39]. The anti-bactericidal activity of chitosan is related to its cationic character with high affinity for the microbial cell wall [28,38,40–42].

Hyaluronic acid (HA) is a natural biopolymer found in all living organisms; the major component in the extracellular of many organs in all body fluids as eyes, skin, joints and hyaline cartilage [43,44]. HA has various applications especially in medical field, it can be used as tumor marker in different types of cancer such as prostate and breast cancer. Also, it's used in wound healing repair and improve the biomechanical properties of tissues. HA interact with many cell surface receptor as CD44, and ICAM-1 causing cellular processess including morghogensis, wound repair, inflammation and metastasis [45–47]. Furthermore, HA has a main role in the viscoelasticity of bio-fluids, it can control the hydration of tissue and transport of water [48–50]. The characteristic of hyaluronic acid as the biocompatibility and the hydrophobicity make it an excellent component in cosmetics [50]. Nowadays HA has been used as drug delivery agent in various fields as ophthalmic, topical and parental [51].

This paper describes the preparation of novel wound dressing sheets based on chitosan/hyaluronan/nonwoven fabrics and their physical, chemical and biological properties were evaluated.

## 2. Experimental

### 2.1. Materials

Chitosan (CS) was isolated from shrimp shell of Brazilian Atlantic Ocean obtained from VCI Brasil Indústria e Comércio de Embalagens Ltda. (Brazil). Citric acid (CTA) was purchased from Sigma Aldrich (Germany) and sodium hyaluronate (HA) high molecular mass (1.5–1.7 MDa, determined by SEC-MAALS) was purchased from Contipro Biotech Ltd., Dolni Dobrouč, Czech Republic. Viscose (100-%, 40 g/m$^2$) nonwoven cotton fabrics and samples (produced by random distribution for web and spun bonded thermally) were used in this study and kindly provided from El-Nasr Company for Spinning, Weaving and Dyeing–El-Mahalla El-Kubra, Egypt. These fabrics were cut into identical sheets (10 × 10 cm) and used for all experiments as described below.

### 2.2. Isolation of chitosan from shrimp shell

Pure chitosan (CS) was isolated from Brazilian Atlantic ocean according to our previous work with small modification [28]. Briefly, shrimp shells were washed with water, acetone and isopropyl alcohol to remove sand, salts and other impurities. Washing was followed by three classical steps: the first step was demineralization which was carried out by 5-% hydrochloric acid solution for 2 h; the second step was deproteinization step (DP) which was done by the use of 5-% aqueous sodium hydroxide for 20 h; finally, the crude chitin was stirred in 50-% sodium hydroxide for 3.5 h to obtain pure chitosan (deacetylation step). The isolated chitosan was fully characterized by FTIR, X-ray diffraction, NMR, TGA-DTG and elemental analysis. The deacetylation degree of chitosan was 95% [28] (determined by NMR and FTIR-ATR techniques).

#### 2.2.1. Immobilization of chitosan citrate/hyaluronan onto nonwoven cotton fabrics

Cotton nonwoven fabrics (NWFs) were used as substrates for immobilization of chitosan citrate (CSCTA) and sodium hyaluronate (HA). Firstly, cotton nonwoven fabrics were washed with hot water for 30 min then with 0.1% sodium bicarbonate to activate the fabric surface. The nonwoven fabrics (10 × 10 cm) were immersed in chitosan citrate (CTA was 5 wt.%) of 0.5, 1, 2 wt.% solutions for 30 min, then squeezed at constant pressure using padding machine. The chitosan citrate/nonwoven fabrics with different concentration of chitosan (0.5, 1, 2 wt.%) (coded as $CS_{0.5}$CTA/NWFs, $CS_1$CTA/NWFs and $CS_2$CTA/NWFs) were dried at 80 °C for 10 min by-thermal fixation machine. The CSCTA/NWFs were dried at 80 °C for 10 min in thermal fixation machine. The CSCTA/NWFs were rinsed for three times with ultrapure water to remove nonattached (non-bonded) materials from the surface of nonwoven sheets and then dried at 80 °C for 10 min. The washing step was repeated three times to obtain layers of chitosan citrate onto non-woven cotton fabrics (CSCTA/NWFs).

The CSCTA/NWFs (coded as $CS_{0.5}$CTA/NWFs, $CS_1$CTA/NWFs, $CS_2$CTA/NWFs) were immersed in the second bath contained 0.5 wt.% of sodium hyaluronate (HA), then $CS_{0.5}$CTA/HA/NWFs, $CS_1$CTA/HA/NWFs, $CS_2$CTA/HA/NWFs were dried at 80 °C for 10 min, rinsed with demineralized water to remove non-attached (non-bonded) materials. The washing step was repeated three times obtained layer of hyaluronan onto chitosan citrate layers. Keep the wet pick up 100% in immersing step in each step. All non-woven fabrics were dried at 150 °C for 5 min. The CSCTA/HA/NWFs were code as $CS_{0.5}$CTA/HA/NWFs, $CS_1$CTA/HA/NWFs and $CS_2$CTA/HA/NWFs.

### 2.3. Wound dressing characterization

Attenuated total reflectance fourier transforms infrared spectroscopy (ATR-FTIR) was performed by the spectrophotometer Nicolet Impact 400 D FTIR-ATR (Nicolet CZ, Prague, Czech Republic) equipped with a ZnSe crystal for the ATR-FTIR spectroscopy. Absorbance was measured as a function of the wavenumber (cm$^{-1}$) between 4000 cm$^{-1}$ and 650 cm$^{-1}$ with the resolution of 8 cm$^{-1}$ and the number of scans equal to 128. Thermal decomposition was measured on thermogravimetric TG, Netzsch 209F3 instrument ($Al_2O_3$ crucible) with the heating rate 10 °C min$^{-1}$ (with data collecting rate 40 points per Kelvin), which was performed in the dynamic nitrogen atmosphere with the pressure of 0.1 MPa. The sample mass for TGA was about 0.9 mg, TGA temperature range 25–800 °C. The morphology of nonwoven fabrics was studied by the scanning electron microscopy (SEM). The nonwoven dressing sheets were cut with a razor scalpel after being frozen in liquid nitrogen for 10 min. X-Ray diffraction (XRD) data were collected utilizing the D8 Advance diffractometer (Bruker AXS, Germany) with Bragg-Brentano θ-θ goniometer (radius 217.5 mm) equipped with a secondary beam curved graphite mono-chromator and Na(Tl) I scintillation detector. The generator was operated at 40 kV and 30 mA and the scan was performed at room temperature from 2 to 50° (2θ) in 0.02° steps with a counting time of 8 s per step.

The antibacterial activity of the wound dressing nonwoven fabrics was performed as follow [54,55]; 1 cm of each wound dress-