International Journal of Biological Macromolecules xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Different chemical groups modification on the surface of chitosan nonwoven dressing and the hemostatic properties

Dong Yan [a], Shihao Hu [a], Zhongzheng Zhou [a], Shah Zeenat [a], Feng Cheng [b], Yang Li [a], Chao Feng [a], Xiaojie Cheng [a,*], Xiguang Chen [a,*]

[a] College of Marine Life Science, Ocean University of China, 5 Yushan Road, 266003 Qingdao, PR China
[b] Center Blood Station of Qingdao, 9# Longde Road, 266071 Qingdao, PR China

ARTICLE INFO

Article history:
Received 25 April 2017
Received in revised form 6 July 2017
Accepted 5 September 2017
Available online xxx

Keywords:
Chitosan nonwoven
Surface modification
Hemostatic property

ABSTRACT

The hemostatic properties of surface modified chitosan nonwoven had been investigated. The succinyl groups, carboxymethyl groups and quaternary ammonium groups were introduced into the surface of chitosan nonwoven (obtained NSCS, CMCS and TMCS nonwoven, respectively). For blood clotting, absorbance value (0.105 ± 0.03) of NSCS1 nonwoven was the smallest (CS 0.307 ± 0.002, NSCS2 0.148 ± 0.002, CMCS1 0.195 ± 0.02, CMCS2 0.233 ± 0.001, TMCS1 0.191 ± 0.002, TMCS2 0.345 ± 0.002), which indicated the stronger hemostatic potential. For platelet aggregation, adenosine diphosphate agonist was added to induce the nonwoven to adhered platelets. The aggregation of platelet with TMCS2 nonwoven was highest ($10.97 \pm 0.16\%$). Further research of blood coagulation mechanism was discussed, which indicated NSCS and CMCS nonwoven could activate the intrinsic pathway of coagulation to accelerate blood coagulation. NSCS1 nonwoven showed the shortest hemostatic time (147 ± 3.7 s) and the lowest blood loss (0.23 ± 0.05 g) in a rabbit ear artery injury model. These results demonstrated that these surface modified chitosan nonwoven dressings could use as a promising hemostatic intervention, especially NSCS nonwoven dressing.

1. Introduction

Hemorrhage in trauma has associated with the increased mortality rate, which is a leading cause of the majority of deaths on the battlefield and civilian settings as well [1,2]. In common condition, the blood coagulation cascade process of the body could occur following these procedures. Initially, platelets are activated and aggregated and adhere to the exposed subendothelial matrix. Plasma proteins and small molecules are released to form a bridge and generated an initial hemostatic plug to reduce the loss of blood. Subsequently, a series of coagulation cascade are triggered to form fibrin clot which reinforce the platelet plug to heal the wound [3,4]. However, the severe or uncontrollable hemorrhage cannot be stopped by the body's natural clotting mechanism, which results in the requirement of a hemostatic intervention.

Currently, Synthetic or natural hemostatic agents including bandage, powder, sponge and gel have been applied to control hemorrhages. Among them, chitosan, a natural polymeric material which derived of deacetylated chitin, that has the excellent properties such as biocompatibility, biodegradability, antimicrobial properties, non-toxicity, and so on [5–7]. The hemostatic property of chitosan is owing to the electrostatic interaction with negatively-charged cell membranes of erythrocytes and platelet, which distincts from the body's natural clotting mechanism [8,9]. Therefore, chitosan has been widely used as hemostatic agent and wound dressing with multiple forms, such as film, hydrogel, sponge, fiber and nonwoven which is spun by fibers [6,10,11]. Among these forms, fiber or nonwoven is the main form because it allow gaseous exchange and be removed easily. Although numerous studies about the hemostatic capacity of chitosan have been reported, there still are limitations including insolubility and weak antibacterial activity which make it an inappropriate therapy [12,13]. Therefore, the surface chemically modified chitosan fibers by processes such as carboxymethylation [14], quaternization [15], succinylation [16], have been investigated to improve the water solubility and antibacterial activity to use as wound dressings. But few studies have been conducted to investigate the hemostatic property of these surface modified chitosan fibers.

In the present work, the chitosan nonwoven was chosen to use as raw material because of the better mechanical property and

* Corresponding authors.
E-mail addresses: chengfeng012400@126.com (F. Cheng), xjcheng@ouc.edu.cn (X. Cheng), xgchen@ouc.edu.cn (X. Chen).

http://dx.doi.org/10.1016/j.ijbiomac.2017.09.008
0141-8130/

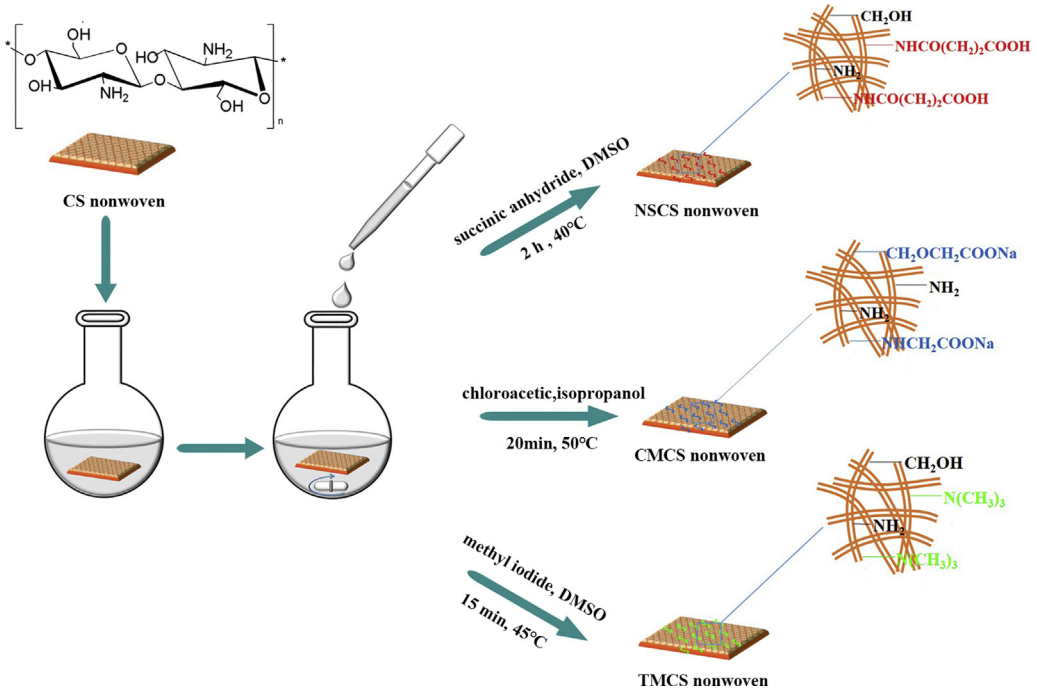

Fig. 1. Different chemical groups modification on the surface of chitosan nonwoven dressing, CS, NSCS, CMCS and TMCS nonwoven.

then NSCS, CMCS and TMCS nonwoven were prepared. To study the hemostatic effect, the different degree of substitution (DS) of nonwoven samples varied by regulating reaction conditions, characterizations such as chemical structure, blood compatibility and cytotoxicity were investigated. In the following work, nonwoven samples were evaluated in a rabbit ear artery injury model. The results suggested that the NSCS, CMCS and TMCS nonwoven exert a better hemostatic property than CS nonwoven.

2. Materials and methods

2.1. Materials

Chitosan nonwoven (CS nonwoven, viscosity = 1092 cps, degree of deacetylation = 91.5%, linear-density = 3.1 cN) were purchased from Weifang Yingke Group Co., Ltd. (Shandong, China). Succinic anhydride was purchased from Yuanhang Chemical Co. Ltd (Tianjin, China). Chloroacetic acid was got from Tianjin Kaixin Chemical Industry Co., Ltd. Methyl iodide (CH_3I), dimethyl sulfoxide (DMSO) and sodium hydroxide (NaOH) were obtained from Qingdao Yunshan Biotechnology Co., Ltd. Rabbits were purchased from Qingdao Institute for Drug Control. All other chemicals used in this work were of analytical grade.

2.2. Preparation of nonwoven dressings

NSCS, CMCS and TMCS nonwoven were synthesized through the modification on the surface of chitosan nonwoven [15–17]. Briefly, 1.0 g CS nonwoven was added into a mixture solution (0.75 g/1.5 g succinic anhydride and 50 mL DMSO) and stirred for 2 h at 40 °C. 1.0 g CS nonwoven, 1.35 g sodium hydroxide, 40 mL of isopropanol and water (4:1 v/v) were added to a flask to swell and alkalize. After 1 h, 0.5 g/1 g chloroacetic acid dissolved in isopropanol was slowly added to the solution and reacted at 50 °C for 20 min 1.0 g CS nonwoven was placed in a round-bottom flask with 40 mL of DMSO and 6 mL of 15% (w/v) NaOH solution for 10 min, subsequently, 1.5 mL/2 mL of methyl iodide was added and oscillated for 15 min at 45 °C. Thereafter, all nonwoven dressings were washed with 75% (v/v) ethanol and then dried.

2.2.1. The degree of substitution of nonwoven

The DS of the NSCS, CMCS and TMCS nonwoven were measured by conductivity titration [18].

2.2.2. The fourier transform infrared spectroscopy (FTIR)

The chemical structure of nonwoven was analyzed using a Fourier transform infrared spectrophotometer. (NEXUE470, Nicolet, Madison, USA).

2.2.3. ^1H NMR spectroscopy

^1H NMR spectrum of CS, NSCS, CMCS and TMCS nonwoven were recorded on a Bruker AV300 instrument and dissolved in the mixed solvent DCl/D_2O, respectively.

2.3. Whole blood clotting test

The blood clotting test was described by Ong et.al [19]. and Leslie W. Chan et.al [2]. Nonwoven samples (1 cm × 1 cm) were placed in glass dishes and kept the temperature to 37 °C. 100 μL of citrated whole blood was pipetted onto each nonwoven dressings followed by the addition of 10 μL of 0.2 M $CaCl_2$ solution and then incubation at 37 °C for 5 min. Each dressing was then placed in a 50 mL of centrifuge tube containing 12.5 mL of distilled water. The tubes were inverted three times to rinse the unclotted blood cells, and the absorbance of the hemoglobin was measured at 540 nm by UV–vis spectrophotometer (UV-1200 MAPADA, China). Then the nonwoven samples were washed with phosphate buffer solution (pH 7.4) several times and immobilized with 2.5% glutaraldehyde for 2 h, dehydrated with a series of ethanol and dried with critical point drier, and then observed by SEM (JSM-6010LA, JEOL Ltd., Japan).

2.4. Platelet aggregation

Blood was collected from the heart of rabbits under anesthesia by a syringe and added into a sodium citrated tube with anti-