Materials Science and Engineering C 70 (2017) 287–295

Contents lists available at ScienceDirect

Materials Science and Engineering C

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

Covalently antibacterial alginate-chitosan hydrogel dressing integrated gelatin microspheres containing tetracycline hydrochloride for wound healing

Huinan Chen [a], Xiaodong Xing [a,*], Huaping Tan [b,**], Yang Jia [b], Tianle Zhou [b], Yong Chen [c], Zhonghua Ling [c], Xiaohong Hu [d]

[a] School of Chemical Engineering, Nanjing University of Science and Technology, Nanjing 210094, China
[b] School of Materials Science and Engineering, Nanjing University of Science and Technology, Nanjing 210094, China
[c] Department of Orthopaedics, Jinling Hospital, Nanjing 210002, China
[d] School of Material Engineering, Jinling Institute of Technology, Nanjing 211169, China

ARTICLE INFO

Article history:
Received 2 April 2016
Received in revised form 12 August 2016
Accepted 31 August 2016
Available online 3 September 2016

Keywords:
Bacterial infection
Hydrogel
Microspheres
Wound dressing
Antibacterial activity

ABSTRACT

An antibacterial and biodegradable composite hydrogel dressing integrated with microspheres is developed for drug delivery and wound healing. The mechanism of gelation is attributed to the Schiff-base reaction between aldehyde and amino groups of oxidized alginate (OAlg) and carboxymethyl chitosan (CMCS). To enhance antibacterial and mechanical properties, tetracycline hydrochloride (TH) loaded gelatin microspheres (GMs) were fabricated by an emulsion cross-linking method, followed by integrating into the OAlg-CMCS hydrogel to produce a composite gel dressing. *In vitro* gelation time, swelling, degradation, compressive modulus and rheological properties of the gel dressing were investigated as the function of microsphere ratios. With increasing ratios of microspheres from 10 to 40 mg/mL, the composite dressing manifested shorter gelation time and lower swelling ratios, as well as higher mechanical strength. Comparing to other formulations, the gel dressing with 30 mg/mL microspheres showed more suitable stabilities and mechanical properties for wound healing. Also, *in vitro* drug release results showed that the loaded TH could be sustained release from the composite gel dressing by contrast with pure hydrogels and microspheres. Furthermore, powerful bacteria growth inhibition effects against *Escherichia coli* and *Staphylococcus aureus* suggested that the composite gel dressing, especially the one with 30 mg/mL GMs containing TH, has a promising future in treatment of bacterial infection.

1. Introduction

In burn care, *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) are the common bacteria and proliferate rapidly after infection [1,2]. A suitable and effective dressing material with antibiotics can not only overcome this shortcoming but reduce the occurrence of antibiotic resistance of bacteria by controlling the local concentration of antibiotics [2–4]. Recently, biodegradable hydrogels have been widely used in tissue engineering and drug delivery, which can function as injectable scaffolds for osteogenesis, cartilage repair, adipose regeneration and wound healing [5–14]. Dressings cast from hydrogels are developed with the advantages of moist environment, good biocompatibility, the ability of absorbing wound exudates and no adherence with wound-tissue [1,15]. Hydrogel dressings are preferred over other dressing materials because they can avoid the secondary damage when dressing changed. Many natural polymer dressings, developed with chitosan, alginate, collagen and hyaluronic acid, have already been studied in wound healing and tissue regeneration [15–19].

Many methods have been employed for the preparation of natural hydrogels, including chemical cross-linking with agents such as carbodiimide, glutaraldehyde, genipin and adipic dihydrazide [20–24]. However, the chemical cross-linking agents are the major obstacle in the use as injectable *in situ* forming polymer scaffolds due to their toxicity to cells [25,26]. By contrast with chemical methods in the synthesis of hydrogel, the Schiff-base reaction proves to be a non-cytotoxic cross-linking reaction, without the participation of any chemical cross-linking agents [11,27]. Herein, a covalent hydrogel dressing is presented by the cross-linking of amine groups from water-soluble carboxymethyl chitosan (CMCS) and aldehyde groups from oxidized alginate (OAlg) *via* the Schiff-base reaction. Chitosan and alginate are natural polysaccharides with good biocompatibilities and biodegradabilities, which have been widely applied in drug delivery, gene therapy and tissue engineering

* Corresponding author.
** Correspondence to: School of Materials Science and Engineering, Nanjing University of Science and Technology, 200 Xiao Ling Wei St, Nanjing 210094, China.
E-mail addresses: xingxiaodong07@njust.edu.cn (X. Xing), hptan@njust.edu.cn (H. Tan).

http://dx.doi.org/10.1016/j.msec.2016.08.086
0928-4931/

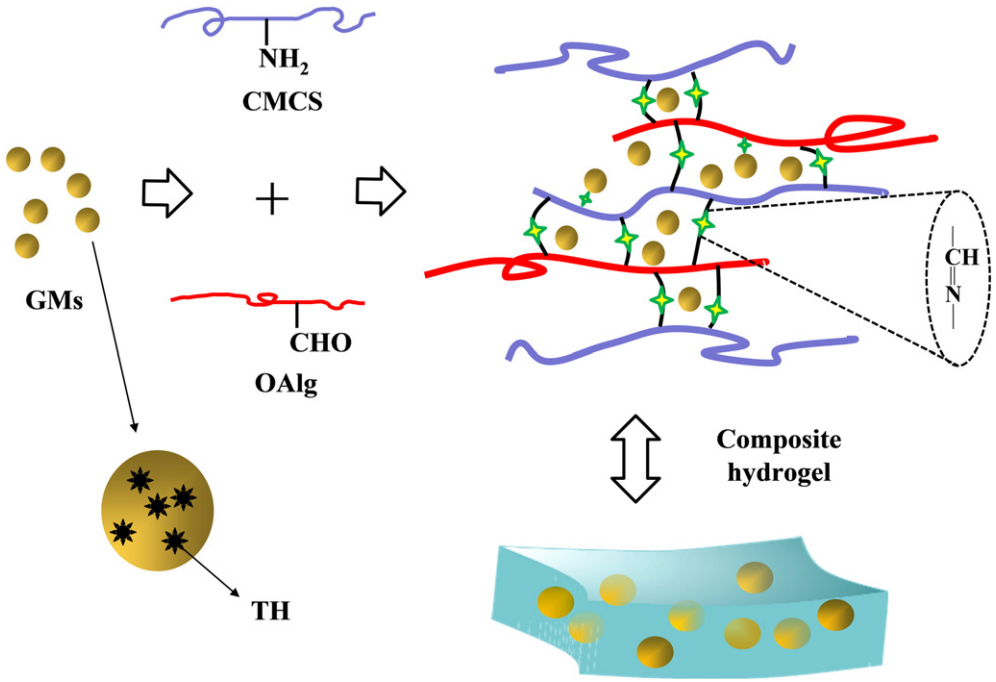

Fig. 1. Reaction schematic illustration of the composite OAlg-CMCS hydrogel (Gel) integrated with gelatin microspheres (GMs) containing tetracycline hydrochloride (TH) *via* the Schiff-base reaction.

[28–32]. Due to their excellent gel-forming properties, CMCS and alginate also show promise in biomedically-relevant hydrogel systems.

In this work, we intend to enhance the performances of natural hydrogels when applied as antimicrobial dressings, especially mechanical properties and drug delivery. We hypothesized that the incorporation of antibiotic-loaded microspheres into gel scaffolds would show expected mechanical properties and sustained drug release. During the past decade, many efforts have been devoted towards fabricating gelatin-based microspheres (GMs) for delivery of cell growth factors and genes to induce tissue regeneration, either in localized or targeted manners [33,34]. Due to the ability of controlled release, GMs have been widely adopted as carriers to immobilize or encapsulate cell growth factors for enhancing cell proliferation and differentiation. Cell growth factors and bioactive drugs can be not only directly encapsulated into GMs during preparation, but also grafted or coated on surface of GMs. Therefore, tetracycline hydrochloride (TH) loaded GMs were fabricated by an emulsion cross-linking method, followed by integrating into OAlg-CMCS hydrogels to produce a composite gel dressing. The effects of varying the ratio of microspheres on gelation time, microstructure, morphology, equilibrium swelling, compressive modulus and degradation *in vitro* were examined. Furthermore, the antibacterial properties against *E. coli* and *S. aureus* were also evaluated in order to examine the positive effects in treatment of bacterial infection.

2. Experimental section

2.1. Materials

Sodium alginate and carboxymethyl chitosan (CMCS) were supplied by Sinopharm Chemical Reagent Co., Ltd. (China). Gelatin was purchased from Tianjin Kemiou Chemical Reagent Co., Ltd. (China). Tetracycline hydrochloride (TH), glutaraldehyde, ninhydrin, *t*-butyl carbazate and sodium periodate were purchased from Aladdin Industrial Corporation (Shanghai, China). All chemicals and reagents were used as received.

2.2. Synthesis of oxidized alginate (OAlg)

Sodium alginate (3 g) was dissolved to 150 mL ultrapure water under stirring to obtain a solution of 2% (w/v) concentration. An aqueous solution of sodium periodate (3 g, 10 mL) was added dropwise into the alginate solution and stirred for 24 h at room temperature in

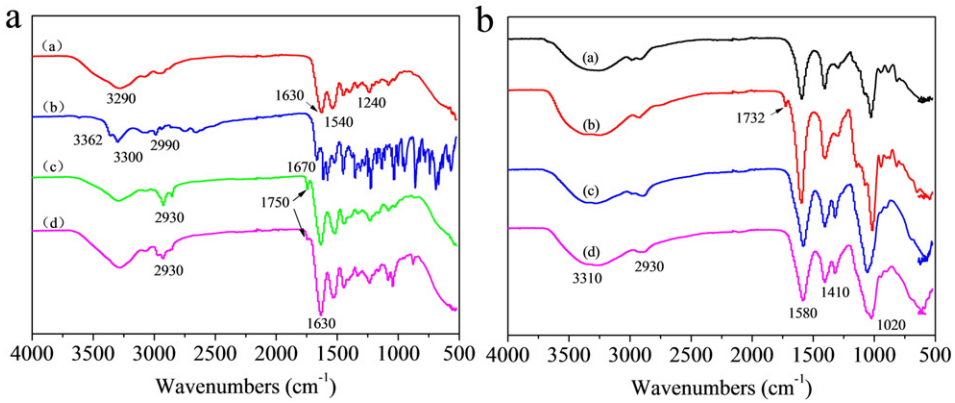

Fig. 2. (a) FTIR spectra of a: gelatin, b: TH, c: GMs, d: TH-loaded GMs (TH/GMs). (b) FTIR spectra of a: alginate, b: OAlg, c: CMCS, d: Gel.