Materials Science and Engineering C 33 (2013) 588–595

Contents lists available at SciVerse ScienceDirect

Materials Science and Engineering C

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

Silver sulfadiazine loaded chitosan/chondroitin sulfate films for a potential wound dressing application

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ARTICLE INFO

Article history:
Received 1 April 2012
Received in revised form 6 August 2012
Accepted 28 September 2012
Available online 6 October 2012

Keywords:
Chitosan
Chondroitin sulfate
Silver sulfadiazine
Antibacterial activity
Wound dressing

ABSTRACT

Silver sulfadiazine (AgSD) loaded chitosan/chondroitin sulfate (CHI/CS) films were formed to be applied as a potential wound dressing material. The liquid uptake capacity of both, CHI/CS and CHI/CS/AgSD, films exhibited a pH-dependent behavior. Tensile tests showed that the amount of CS used to form the films and the further incorporation of AgSD affect the mechanical properties of the films. In vitro AgSD-release assays showed that the CHI/CS mass ratio influences the AgSD release rate. All the investigated CHI/CS/AgSD films sustain the AgSD release up to 96 h at physiological pH. Antibacterial activity and cell viability assays showed that all the CHI/CS/AgSD films have activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus* but they were not toxic to *Vero* cells. The results presented in this work indicate that the CHI/CS/AgSD exhibits potential to be applied as a wound dressing material.

1. Introduction

The human skin is the first and main barrier against the invasion of external agents harmful to our body, but in some cases, it may be injured or even destroyed, as in the case of severe burns. When the skin suffers burning, the proteins that constitute the skin are degraded, and the decreasing in the oxygen provision provides an excellent medium for the development and proliferation of pathogenic microorganisms [1–4]. Furthermore, the vascular obstruction by thermal injuring of vessels hinders the transport of antibiotics and fluids of the immune system to the burned area. One of the treatments for severe injuries caused by burns is to remove a thin layer of healthy skin from the patient's body and graft it into the damaged region. To protect the skin from infections and dehydration during the period between hospital admission and the grafting chirurgical procedure, a suitable coating should temporarily cover the skin wound. Such procedure has been used, at least, for two decades [5–9]. For this purpose many polymeric membranes and films have been developed from either synthetic, such as polyurethane, polyethylene, polycaprolactone, poly(lactic acid), silicone rubber, and natural polymers, such as alginate, chitosan, gelatin and collagen [10–15]. Among the several manners and techniques applied to obtain polymeric materials with good properties and satisfactory performance, the polyelectrolyte complexation could be highlighted [16–18]. The interactions between macromolecules with opposite charges occur in mild conditions, resulting in materials with hydrophilic character and responsive to some properties (such as pH, ionic strength, etc.) [19,20]. The properties of polyelectrolyte complexes are determined by the strong interaction between the opposite-charged macromolecules, being more important than those when strong electrostatic interactions are evolved [21].

In recent years, many works have reported the formation of polyelectrolyte complexes based on chitosan (CHI) and other polymeric species and their potential for biomedical, pharmaceutical and drug delivery applications [22–24]. CHI is a well-known linear copolymer derived from the biopolymer chitin that shows interesting and intended properties, like biocompatibility, low toxicity, haemostatic potential, anti-infectious activity, and biodegradability [19,25,26]. Other particular properties presented by CHI are action against tumors and anticoagulant property allowing its application as biomaterials in drug delivery systems, tissue repair and regeneration [27,28]. CHI also shows a cationic nature and a high charge density in acid environment enabling the formation of polyelectrolyte complexes with polyanionic polymers [29]. Chondroitin sulfate (CS) a biopolymer belonging to the class of glycosaminoglycans, GAGs, and a structural component of the cartilaginous matrix, shows a great negative charge density making it an optimal candidate to form a polyelectrolyte complex with CHI [30].

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http://dx.doi.org/10.1016/j.msec.2012.09.025

This work reports on the formation of CHI/CS films by polyelectrolyte complexation. The CHI/CS mass ratio was varied to verify possible changes in the final properties of the films (physical and morphological). Furthermore, to improve the applicability of the CHI/CS films as wound dressing, silver sulfadiazine, AgSD, was incorporated in the film formulation. AgSD is widely used as an effective antibiotic for injuries caused by burns [31]. In addition, AgSD is used as a model drug against the bacterial activity in the treatment of infected wounds [32].

2. Materials and methods

2.1. Materials

CHI with deacetylated degree equal to 85% and viscometric mass (M_V) equal to 87×10^3 g mol^{-1}, was purchased from Golden-Shell Biochemical, China. CS (kindly supplied by Solabia, Brazil) presented M_V equal to 22×10^3 g mol^{-1}. Both M_V were obtained according to methodology previously described by Fajardo et al. [29]. AgSD was purchased from Siddharth International, India. All reactants, of analytical grade, were used without further purification.

2.2. CHI/CS film formation

The CHI/CS films were formed by solvent casting method. Briefly, CHI (2 wt.%) was solubilized in acid acetic solution (1.5 wt/vol.%) and CS (8 wt/vol.%) was solubilized in distilled water. The mixing of the CHI and CS solutions formed the films. After mixing the solutions, each one was homogenized under magnetic stirring and then poured into Petri dish (polystyrene round-plate shape — diameter of 85 mm and height of 10 mm). Using three different CHI/CS mass ratios (wt/wt.%) 40/60, 50/50 and 60/40 the as-formed films were labeled as MCS1, MCS2 and MCS3, respectively. After this, the films were stored in oven at 40 °C for 24 h. The dry films were peeled off from the Petri dishes and immediately soaked in NaOH solution (0.2 M); purified in distilled water overnight and then dried at room temperature. The procedure used to form the CHI/CS/AgSD films was similar to that described above, but during the CHI solubilization step for each film sample 15 mg of AgSD (mass ratios between CHI/CS films and AgSD were around 100:1) was added. The CHI/CS/AgSD films were labeled as MCS1-1, MCS2-2 and MCS3-3, respectively. All the films were characterized by Fourier Transformer Infra Red spectroscopy (FTIR) and Scanning Electronic Microscopy (SEM).

2.3. Film characterization: FTIR analysis, swelling degree and mechanical properties

The as-formed films (loaded and non-loaded) were characterized by infrared spectroscopy technique using a transform infrared spectrophotometer (Shimadzu Scientific Instruments, Model 8300, Japan), operating in the region from 4000 to 500 cm^{-1}, resolution of 4 cm^{-1}. In addition, pure CHI, CS, and AgSD were also characterized by the FTIR technique.

The liquid uptake capability of each film was evaluated through the swelling degree parameter (S), which is calculated through the following Eq. (1): [29]

$$S = \frac{W_{it} - W_o}{W_o} \quad (1)$$

where W_{it} is the weight of sample swollen at time t and W_o is the weight of dry sample.

For evaluating the liquid uptake capacity at different pHs, the previously weighed dry films were immersed in buffer solutions, pH 2, 7 and 10. The buffer solutions, at constant ionic strength (0.1 mol L^{-1}, by the use of KCl), were produced according to the National Book of Formulas — United States Pharmacopoeia (USP 30-NF25) [33]. Therefore, at certain time intervals (1, 3, 6, 12, 24, and 48 h, respectively) after the immersion, each sample was removed and weighed, in order to determine the S value. For each condition, three samples (n = 3) were used, so the S data refer to the average of triplicates.

The mechanical properties of the swollen films were evaluated by tensile tests in a Texturometer equipment (Stable Microsystems, Model TA.XT2, England) according to the ASTM D882 method. For the tensile tests, all the films were cut in small rectangular body proofs (50 mm × 15 mm). The tests were performed at controlled temperature (25 °C) and relative humidity (50%). The tensile parameters determined were tension of rupture (T_r) and Young's modulus. These parameters were calculated according to the following Eqs. (2) and (3):

$$T_r = \frac{F_{max}}{A} \quad (2)$$

$$\text{Young's Modulus} = \frac{F_{max} \times L_1}{A \times (L_2 - L_1)} \quad (3)$$

where F_{max} is the force necessary to break the body proof (N), A is the area (m^2) of the transversal section of the samples, L_1 are the samples' initial length (mm) and L_2 is the samples' length (mm) before the rupture point.

2.4. In vitro AgSD-release assays

The in vitro AgSD release assays from the loaded films were performed using film samples (dry weight 0.1 g), which were immersed in 50 mL of phosphate buffered saline solution, PBS, pH 7.4 with controlled temperature (37 °C). In desired time intervals, aliquots of supernatant were withdrawn to determinate the amount of AgSD released from the loaded films. The aliquots were analyzed in a UV–vis spectrophotometer (Femto, model 800Xi, Brazil) at 430 nm. After the UV analyses the withdrawn aliquots were added back to maintain the volume constant. The amounts of AgSD-released were quantified from a previously built analytical curve. The analytical curve was designed from the plot of absorbance versus standard AgSD solutions with known concentrations varying from 5.00×10^{-5} to 3.125×10^{-6} mg L^{-1} using PBS as solvent. The linear correlation coefficient (R^2) value was as high as 0.999. All the experiments were done in triplicates (n = 3).

The modifications in the film morphology promoted by the variation of the CHI/CS mass ratio during the film formation, by AgSD incorporation and by AgSD release process were investigated through SEM images. The samples' surfaces were sputter-coated with a thin layer of gold for allowing the SEM visualization and the images were taken by applying an electron accelerating voltage of 10 keV.

2.5. Antibacterial activity assays

The antibacterial activity of the AgSD loaded CHI/CS films was evaluated through the determination of their capability in inhibiting the growth of *Pseudomonas aeruginosa* (*P. aeruginosa* — ATCC 27853) and *Staphylococcus aureus* (*S. aureus* — ATCC 25923) strains. For these tests disks of the loaded films (diameter 5 mm) were put into the inoculation medium in agar plates, as well as the disks of the non-loaded films (used as control). The agar plates were kept under controlled temperature (37 °C) for 24 h. After this, the inhibition zones, which are the diameter of the inhibitory circles, around the loaded films were compared to the not AgSD loaded films.

2.6. Cell viability assays

The cytotoxicity of CHI/CS and CHI/CS/AgSD films was evaluated through the viability of *Vero* cells using sulforhodamine B (SRB). For