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Preparation, characterization and biocompatibility of poly(vinyl alcohol) films containing tetracycline hydrochloride-loaded quaternized chitosan nanoparticles

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ABSTRACT

Poly(vinyl alcohol) films are widely used in biomaterials applications due to their excellent chemical and thermal stability, transparency, biocompatibility, nontoxicity, and good film-forming ability. In this study, tetracycline hydrochloride (TC) has been encapsulated in quaternized chitosan nanoparticles (QChNPs) by ionic gelation of QCh with sodium tripolyphosphate (TPP). The success of the encapsulation was confirmed by Fourier transform infrared (FT-IR) spectroscopy. The obtained nanoparticles exhibited a regular distribution and spherical shape, with a size range of 450–800 nm as observed by scanning electron microscopy (SEM). The encapsulation efficiencies (EE) of the TC-loaded QChNPs were approximately 72–95%. The obtained nanoparticles were then added into a PVA solution to fabricate PVA films containing TC-loaded QChNPs. In vitro release studies showed an initial burst effect followed by a slow drug release. The antibacterial activities of the PVA films containing TC-loaded QChNPs against *E. coli, S. Aureus*, and *Ent. faecium* were evaluated systematically by the disk diffusion method (AATCC 147) and broth dilution method. Finally, the indirect cytotoxicity of the wound dressings was studied in mouse fibroblast (L929) and human fibroblast cells (FB cells) using an MTT assay.

1. Introduction

After there is damage to skin, the wound healing process begins. The entire process of wound healing is a complex and ordered cascade of events, which can be divided into five phases of hemostasis, inflammation, migration, proliferation, and maturation [1–3]. Currently, a wide range of physical products are available commercially for use as wound dressing and healing, including hydrofibers, hydrogels, foams, and films [4, 5]. Moreover, an effective wound dressing should provide a moist environment with biocompatibility and be non-toxic, non-allergenic, non-adherent, and prevent bacterial infection [6–8].

Among various polymers, the use of blends of poly(vinyl alcohol) (PVA) and chitosan (CS) have been developed to combine the excellent properties of both polymers for many applications, such as food packaging [9], tissue engineering [10], drug delivery systems [11], and wound dressings [12]. PVA is a water-soluble synthetic polymer. Its outstanding properties include excellent chemical and thermal stability, transparency, biocompatibility, nontoxicity, and good film-forming ability. In addition, CS is a natural polysaccharide that is non-toxic and has antimicrobial properties Duttaetal.2009. [13] reviewed the use of CS based antimicrobial films in food applications, and the results showed that the physical properties of CS films can be improved by blending with PVA. Moreover, chitosan blend films can be incorporated with other substances (e.g., silver nanoparticles, essential oils) to improve features such as antimicrobial activity or antioxidant activity [13, 14].

However, the applications of chitosan have been limited because its solubility; it is only soluble in acidic media, and it starts to lose

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its antibacterial activity at pH values higher than 6.5. Therefore, water-soluble chitosan derivatives that are soluble in a wide range of pH values have been used to overcome these limitations [15, 16]. Among chitosan derivatives, quaternary chitosan (QCh), a chitosan derivative with quaternary ammonium groups, has excellent antibacterial and antifungal activity [17–19]. Blends of PVA/QCh films have been prepared by simple mixing and casting methods, and their antibacterial activity against gram-negative (*E. Coli*) and gram-positive (*S. aureus*) bacteria have been studied. The study results summarized that the blend films were perfectly compatible and miscible polymers via hydrogen bonding interactions between the hydroxyl groups and showed notable activity against *S. aureus* and *E. coli* [20].

During recent years, the potential of nanoparticles as drug delivery systems has been shown in numerous studies. Chitosan and its derivatives have been used to encapsulate drugs, including essential oils [21], vaccines, [22] vitamins [23], and proteins [24], for the pharmaceutical and biomedical fields owing to its excellent biocompatibility, nontoxicity, mucoadhesive properties and good drug permeability [25]. The most commonly used technique by far for nanoparticle preparation from chitosan derivatives is ionotropic gelation [26]. The particles can be easily obtained via an ionic gelation process using electrostatic interaction between the charges of the polycation of chitosan and an anionic substance. Sodium tripolyphosphate (TPP) has been widely used as an anionic crosslinker [23, 24, 26, 27].

Tetracycline HCl (TC) is a group of broad spectrum antibiotics used against many gram-negative and gram-positive bacteria [28–30]. Due to the sensitivity to sunlight of TC, which consequently limits the efficiency of the drug, a carrier is required for successful targeted drug delivery [31, 32]. A recent report has suggested the usage of tetracycline as a drug loaded into bacterial cellulose that could be released in a sustained manner, displaying a steady release after an initial burst release and effective antibacterial activity [33].

In this work, we have prepared tetracycline hydrochloride-loaded quaternary ammonium chitosan nanoparticles (TC/QChNPs) via the ionic gelation method. The obtained nanoparticles were characterized for size, shape, zeta-potential and encapsulation efficiency (EE). Then, PVA films containing TC/QChNPs were fabricated via a solution casting method for use as a wound dressing. The *in vitro* release and antibacterial activities against *E. coli* and *S. aureus* were investigated. In addition, the indirect cytotoxicity of the wound dressings were studied in mouse fibroblast (L929) and human fibroblast cells (FB cells) by using MTT assay.

2. Materials and methods

2.1. Materials

Chitosan (CS) (medium molecular weight, 75–85% degree of deacetylation); poly(vinyl alcohol), 99+ % hydrolyzed; 3-chloro-2-hydroxypropyl trimethylammonium chloride (CHPTAC) solution, 60 wt % in H_2O; sodium tripolyphosphate (TPP); and iodine were purchased from Sigma-Aldrich (USA). Tetracycline hydrochloride (TC) was obtained from SRL Ltd. (India). Tween 80 was purchased from RFCL Ltd. (India). Sodium hydroxide, acetic acid, methanol, ethanol and acetone were purchased from RCI Labscan Ltd. (Thailand). All chemicals were of analytical reagent grade and used without further purification.

2.2. Preparation of neat and TC/QChNPs-loaded PVA films

2.2.1. Quaternary ammonium chitosan

QCh was prepared in a similar manner to that reported by Sajomsang et al. [17]. Briefly, 1 g of chitosan was dissolved in 50 mL of 1% (w/v) acetic acid. Chitosan solution was then added dropwise into 2% (w/v) of Na_2CO_3 in H_2O/MeOH. Next, the regenerated chitosan was recovered by filtration for the further steps. In total, 40 mL of the CHPTAC solution was added to the reaction bottle, and the pH of the solution was adjusted to 8 with NaOH (20% w/v). Then, 0.25 g of iodine was added, together with the regenerated chitosan, into the reaction bottle and stirred for 48 h at room temperature. After that, the distilled water was added and the temperature was raised to 60 °C for 24 h. The solution was dialyzed in distilled water for 48 h. The dialyzed solution was concentrated using a rotary evaporator and precipitated in acetone. Prior to further uses, the product was dried at room temperature overnight under a nitrogen stream.

2.2.2. Tetracycline-loaded quaternary ammonium chitosan nanoparticles (TC/QChNPs)

The TC/QChNPs were prepared based on the ionic cross-linking of QCh with TPP. A total of 25 mg of QCh was dissolved in 10 mL of distilled water. When a homogeneous solution was observed, Tween80 (0.25 g) was mixed into the solution by stirring at 60 °C for 1 h and then cooled down to room temperature. After that, tetracycline was added to the solution (QCh at drug ratios of 1:0, 1:1, 1:2, 1:3, 1:4 and 1:5) and stirred for 20 min. Then, 40 mL of 0.5% (w/v) TPP solution was gradually added (2 mL/min) into the solution, which was allowed to stir for 20 min. After that, the TC/QChNPs were collected by centrifugation at 10,000 rpm for 30 min at 20 °C. A supernatant was collected for further measurement of the encapsulation efficiency. Then, the TC/QChNPs were washed with distilled water several times, redispersed in 10 mL of distilled water and stored at −20 °C. The TC-loaded QChNPs with a drug ratio of 1:5 were selected for a further comparison study of the release characteristics with TC/QChNPs-loaded PVA films.

2.2.3. TC/QChNPs-loaded and TC-loaded PVA films

PVA (5% w/v) was dissolved in 20 mL of distilled water at 90 °C for 4 h and then cooled down to room temperature. After that, the TC/QChNPs suspensions (5 mL) were added in the PVA solution and allowed to stir for 20 min. The homogeneous solutions were then cast onto a petri dish and dried at a temperature of 35 °C for 48 h in a temperature-controlled incubator. For comparison, TC-loaded PVA film was prepared by adding 50 mg of TC in a 20 mL PVA solution and fabricated by the same casting procedure.

2.3. Characterizations

FTIR spectra were measured in transmission mode with a Thermo Nicolet Nexus 670 Fourier transform infrared spectrometer using 32 scans at a resolution of 4 cm^{-1} from 4000 to 400 cm^{-1}. Zeta potential and particle size were measured at 25 °C using a Zetasizer model S4700, Malvern Instruments (UK). The morphologies of QChNPs and TC/QChNPs were observed by field emission scanning electron microscopy (FESEM; JSM-7001F) operating at an accelerating voltage of 10–15 kV. The nanoparticle suspensions were spread on a glass plate, dried at room temperature and then coated with a thin layer of gold under high vacuum conditions. UV–Vis absorption spectra were recorded over wavelengths ranging from 200 to 400 nm by a Shimadzu UV-1800 spectrophotometer (Japan).

2.4. Degree of quaternization

^1H NMR spectra were measured using a Bruker AVANCE 400 MHz spectrometer. All measurements were performed at