Characterization of Wound Dressing with Microspheres Containing Levofloxacin

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Abstract: A novel functional material was prepared by composing chitosan-levofloxacin complex microspheres with viscose nonwoven fabrics. The complex microspheres were made by ionic and emulsion crosslinking method. The morphology and formulation of the material were investigated by SEM，FTIR and DSC. The drug release property in vitro was evaluated by UV. The cytotoxicity and its antibacterial property were tested by L929 fibroblast culture and the spread plate method respectively. The results showed that the microspheres were embedded in the fabrics, chitosan microspheres were formed by Schiff base reaction and there are no chemical crosslinking appearance between viscose fibers and chitosan-levofloxacin complex microspheres. Levofloxacin could release slowly and its sustained release property can be controlled by the concentration of chitosan solution effectively. In addition, there were no obvious toxicity role presented and its antibacterial action was excellent. The functional material has the potential to be used as levofloxacin sustained releasing wound dressing.

Keywords: nonwovens; microspheres; levofloxacin; slow release

1. Introduction

Chitosan is an unbranched binary heteropolysaccharide consisting of the two units N-acetyl-D-glucosamine and D-glucosamine, obtained by partial deacetylation of chitin normally leading to a degree of deacetylation of 70% to 95%. [1]Since the free amino groups on this polymeric chain contribute to the reactive and polycationic nature that exhibit complexation properties, chitosan and its modified analogs have many applications in medicine, biochemical separation systems, biomaterials and drug controlled release systems .[2-4] It is one of the natural polymers that has a high potential on wound healing application. [5,6]The application of skin wound healing needs to immobilize chitosan on their surface of the layer materials, which are usually in the form of film, fabric, sheet, or nonwoven, to enhance the mechanical properties. [7-10] Some methods, such as irradiation and the redox system, combined with some chemical reagents are known in this field to treat the surface of the layer materials to modify the property of their surfaces and increase the immobility of biomaterials on those surfaces. [11-14] Eylem prepared ciprofloxacin releasing system by using alginate and chitosan as skin replacement material to be used in wound healing and burn dressing applications. Highest release rate around 92% total release in 4 h was obtained with the lowest crosslinker concentration. [15] However, due to drug release in short time, wound dressings need to be replaced frequently. Therefore, drug release system for a long time should be prepared on wound dressing.

This airticle mainly focused on the prepared method of chitosan microspheres containing levofloxacin, its combination style with nonwoven fabrics and relative drug release ,cytotoxicity and antibacterial properties. By the incorporation of chitosan and sodium citrate, the in vitro kinetic release of levofloxacin was determined by some factors apparently. Additionally, antimicrobial activity and its biocompatibility were studited.

2 Experiments

2.1 Materials

Nonwoven fabrics were obtained from HuaNuo Inc. Chitosan(Mw=98,000;270,000; DD.=85%) was obtained from aokang Inc., was purified by dissolving in 1% (w/v) acetic acid and filtration, and recovered by neutralizing. Chitosan gel was filtered out with muslin cloth and washed with a copious volume of deionized water until the washings were neutral with pH 7.0. The collected chitosan gel was vaccum-dried for 24h prior to use. Other chemical reagents were all analytical purification.

2.2 Preparation of chitosan microspheres loaded with levofloxacin

Chitosan microspheres were prepared by a novel water-in-oil(W/O) emulsification process along with an ionic coacervation technique. Firstly, suitable amount of levofloxacin and sodium citrate solutions were added to different concentration of chitosan 1%acetic acid solution mixing to form suspension A. The suspension A was agitated at 1500 rpm using a magnetic stirrer. An emulsified oil phase was prepared by mixing 30ml of olive oil

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Contract grant sponsor: Tianjin Natural Science Foundation; Contract grant No.09JCZDJC18800

and 4 ml Span 80 and tween 80(5% V/V) with an overhead stirrer. Then 10ml of suspension A was slowly added to oil phase at stirring speed of 2500 rpm for 2h. Finally, 4% w/v glutaraldehyde was initiated in mixture by dropwise for 30min at 50℃ in water bath. After the cross-linking reaction, the oil phase of the mixture containing chitosan microspheres was slowly decanted and immediately centrifugated. The microspheres obtained was added to 100ml acetone. The washing was repeated with acetone until discrete. The recovered microspheres were dried in vaccum desiccator at 50℃. The microspheres were optimized according to the process variables. The whole process was carried out at room temperature.

2.3 Preparation of wound dressing loaded with chitosan microspheres

The nonwovens (5cm*5cm) were immersed in 4% NaOH solution for 4 hours, washed repeatedly with water until the PH of water was 7. 0. The microspheres prepared according to 2.2 was in ethanol. The pretreated nonwoven were immersed in well-distributed microspheres solution for a period of time, removed and dried in vaccum desiccator at 50℃ for 12h.

2.4 Characteristic of wound dressing

2.4.1 SEM observation of nonwovens loaded with chitosan microspheres

The distribution of the microspheres on the nonwoven samples and the morphous of chitosan microspheres were characterized by SEM (FEI-QUANTA200). Samples were sputter-coated with a layer of Au under argon atmosphere, and 20 and 5 kV acceleration voltages were used.

2.4.2 FTIR analysis of chitosan microsphere containing levofloxacin

Infrared (IR) spectra of samples (chitosan powders; chitosan microspheres; levofloxacin; chitosan microspheres containing levofloxacin) were recorded with a FT-IR spectrophotometer (NICOLET 380,Thermo Electron Co., USA). samples were scanned from 500 to 4000 cm^{-1}.

2.4.3 DSC of nonwovens with microspheres containing levofloxacin

Temperature range of DSC (PERKIN-ELMER DSC7) was from25℃ to 250℃.

2.4 In vitro drug release studies.

1,2,4,8,10,15 and 20μg/ml levofloxacin in PBS solution were prepared to be tested the absorbance at 288nm. The obtained datas were calculated to the standard curve equation of levofloxacin.[16] 20 mm-diameter wound dressing were placed in bag and put into a cell containing 50mL of phosphate buffered saline (pH 7.4, 0.1 M phosphate buffer, 0.9% (w/v) saline)at 32°C to simulate local epidermal. Aliquots (2.0 ml) were withdrawn from these solutions at fixed time intervals of 0.5, 1,3,7,12,24,48,72,120 and 168h to test levofloxacin concentration at 288 nm. Equivalent volumes of fresh phosphate-buffered saline were replaced into the cell after each sampling to maintain constant medium volume. Release studies were carried out in triplicate.

2.5 Antibacterial activity

Nonwoven fabric samples were examined according to AATCC Test Method100-1998. In this study the sterile solutions that specimens soaked for 10min,120min,480min,1440min, were respectively contacted with 2mL solution having 10^9CFU/mL, (CFU=Colony Forming units), for S. aureus to assess their bactericidal activities. the surviving bacteria were counted by the spread plate method. [17]

2.6 Cell viability

L929 fibroblasts were used to assay the cytotoxacity of wound dressing. The cells were thawed from the frozen liquid nitrogen and seeded in the culture medium(80% 1640 Medium, 15%fetal bovine serum) in a incubator at 37 ℃ with 95% air and 5% CO_2. 100μl cell suspension （40,000 cells/ml） was seeded into each cell of 96-well polystyrene plates for 24h. The wound dressing that had been sterilized with Co60 rays should be shaped into 5mm in diameter. The samples were put into culture dish and added 1ml culture medium in each dishes. After all samples were cultured for 24h, the extract liquid and 50% extract liquid were respectively distributed in another 96-well plates with 100μl medium. As reagent control, blank culture medium was negative control and 10%DMSO was positive control material. 96-well plates was put in the incubator under standard culture conditions for 72h, and cell morphous was observed by microscopy ().[18]

3. Results and Discussion

3.1 SEM observation

Figure 1(c) demonstrates SEM photograph of the surface of dressings which medicates the microspheres that were embedded in fabrics. Figure 1(b)indicates that microspheres can be combined firmly with fibers and maintain integrated appearance. The microspheres were embedded in the viscose fibers because of theirs scrambled concave surface. The microspheres were round and smooth and with the diamiters no more than 5μm Figure 1(a) which shows chitosan microspheres could pass through airspace of surface layer fibers.

3.2 FTIR spectrum analysis

Figure 2 shows infrared spectrogram of chitosan microspheres loading levofloxacin and its raw materials. Compared chitosan powders（A）with blank microspheres(B), amide I spectral band of chitosan powders at

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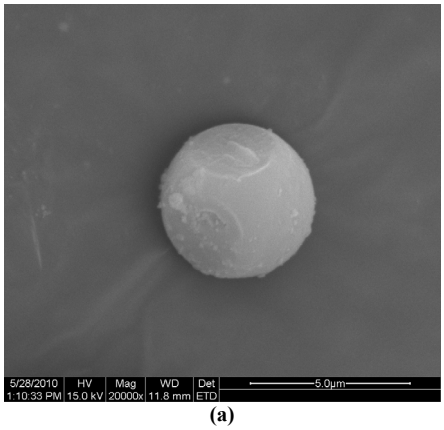
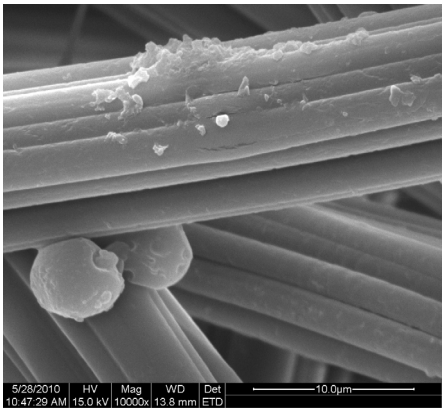
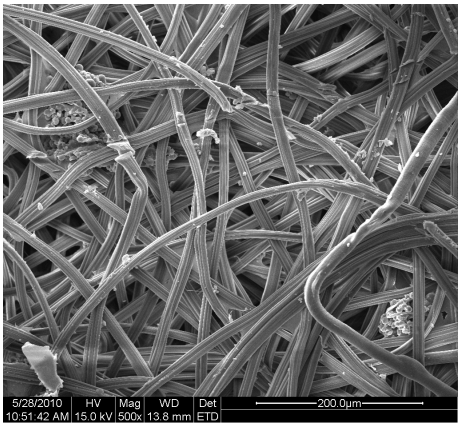
1658cm^{-1} ,spectral band of C=N streching vibration due to amino groups of chitosan and glutaraldehyde generate schiff bases at 1619 cm^{-1} were noted. A new band at 1715 cm^{-1} may be overlapped of amide I spectral band and carbonyl streching vibration that slight COO^- interacted with $-NH_2$ of chitosan. [19]In addition, as described for spectrogram of chitosan microspheres containing levofloxacin(D), the characteristic absorption bands of chitoan and levofloxacin reserved together demonstrates chitosan had no reaction with levofloxacin.



(a)



(b)



(c)

Figure 1. SEM micrograph (a) chitosan microsphere; (b)&(C) the surface of nonwovens with microspheres containing levofloxacin

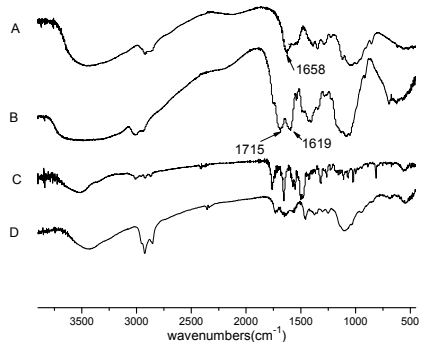


Figure 2. FTIR spectrum A.chitosan powders B. chitosan microspheres C.levofloxacin D.chitosan microspheres containing levofloxacin

3.3 DSC analysis

As is shown by the figure 3 , The endothermal peak of nonwovens Tg at 194℃, and Tg of chitosan microspheres at 125℃, nonwovens embedded into chitosan microspheres have two endothermal peaks, which illustrated no cross linking reaction happened between viscose fibers and microspheres.

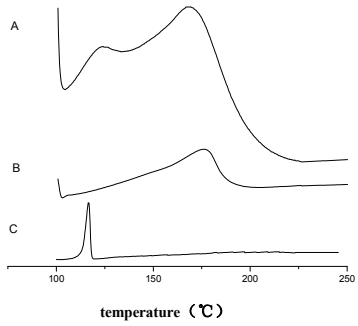


Figure 3. A. Nonwovens loading microspheres B. Non-wovens C.Chitosan microspheres

3.4 Effect of chitosan concentration on levofloxacin accumulate release ratio

By the method, standard curve equation of levofloxacin in PBS solution was measured as y=0.0661x+0.0275(r^2=0.9996), in which y indicates absorbance of levofloxacin in PBS; x indicates concentration of levofloxacin in PBS: r is related factor.

Accumulated release ratios of different chitosan concentrations were shown in Figure 4. When chitosan concentration was 10mg/ml, it's easy to reach 90% of total release amount in 72h, and 50% in 24h. When chitosan concentration was 15mg/ml,well-distributed controlled release curve was revealed before 50% of release amount in 72h. Chitosan concentration was 25mg/ml higher, however, the levofloxacin release velocity accelerated because of 50% of release amount in 36h.The drug diffusion rate was influenced significantly by chitosan concentrations, which was related to the

number of reaction NH_2-terminal on chitosan molecule chain.[20]

3.5 Antimicrobial activity and cytotoxicity analysis

After contacting nonwovens loaded with chitosan microspheres with bacteria suspensions for different periods of time, the bacteria suspensions were spread on the agar medium. Figure 5 shows that the counts of bacterium decreased with drug releasing time, which implied levofloxacin embedded in nonwovens still had bioactivity and its release was progressing.

Cytotoxicity test shows the fibroblast cells could attach、spread and grow with ideal cell morphologies when contacted with sample extracts. The cell morphous of test samples and negative control is nearly shuttle-shaped (Figure 6), which indicates test samples have no toxicity.

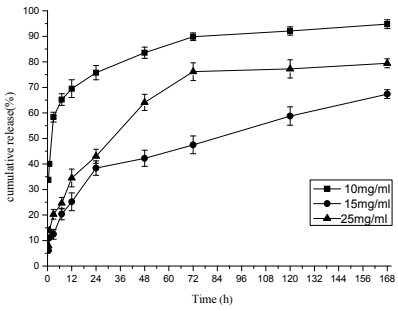


Figure 4. Effect of different chitosan concentrations on cumulative release

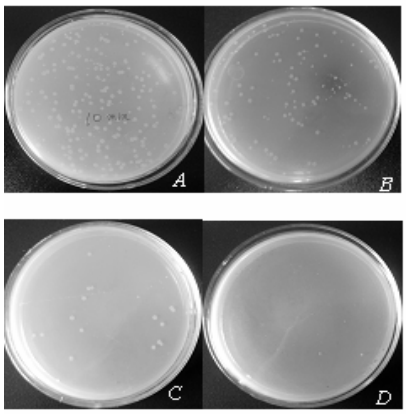


Figure 5. Antimicrobial activity of different levofloxacin release time A .10min; B.120min ; C. 480min; D.1440min

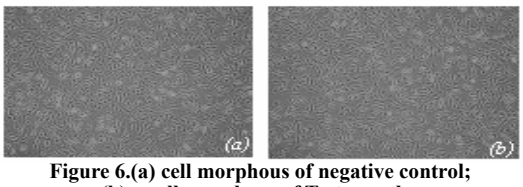


Figure 6.(a) cell morphous of negative control; (b) cell morphous of Test samples

4. Conclusion

A novel wound dressing was fabricated by impregnating viscose nonwovens into chitosan-levofloxacin microspheres. The microspheres could be embedded in fabrics and complexed by physical form. Levofloxacin can be released adjustably by controlling the concentrations of chitosan. Its cytoxicity was minimal and its antibacterial property was excellent. These findings suggest it would be a promising carrier as patch delivery system.

5. Acknowledgment

The authors express their appreciation to Tianjin Natural Science Foundation (Grant No.09JCZDJC18800).

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