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Fabrication and characterization of chitosan film impregnated ciprofloxacin drug: A comparative study

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ABSTRACT

Present study focuses on fabrication of biofilm from chitosan (natural polymer) for loading of the drug to heal the wound. The chitosan based film serves various advantages like biocompatibility, cost effective, easy formulation and sustained drug delivery for wound healing. Three different concentrations of chitosan (Ch) films were prepared like 1% (Ch1), 1.5% (Ch1.5) and 2% (Ch2). These films were impregnated with ciprofloxacin drug and they were characterized by various parameters like thickness, tensile strength, *in-vitro* drug release and swelling properties. The chemical structure and morphology of drug loaded biofilms were analyzed by fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM) analysis and their antibacterial efficacy was also evaluated against bacteria such as *Staphylococcus aureus*, *Escherichia coli and Pseudomonas aeruginosa*. The *in-vitro* biocompatibility was evaluated using NIH 3T3 fibroblast cell lines. The results revels that the ciprofloxacin loaded chitosan biofilm might serve as a potential drug delivery system for wound healing applications.

1. Introduction

The principle objective in wound management is to heal the wound in the shortest possible time, with minimal pain, uneasiness and scarring to the patient and must occur in a physiological environment conducive to tissue repair and regeneration (Weller and Sussman, 2006). In recent years, understanding the importance of moist wound healing has led to the development of hydroactive dressings. Hydro active wound dressings maintain a moist environment at the wound site allowing the wound fluids and growth factors to remain in contact with the wound and thus they fasten up the healing process. Moreover, hydroactive dressings can be peeled off with ease and also they will not cause much disturbance to the newly formed granulation and epithelial tissue (Duncan et al., 2002). The infection will prolong the inflammatory phase and delay the normal wound healing process. So, suitable antimicrobial agent should be incorporate with this hydroactive dressing. This medicated wound dressings possess a defined functionality beyond providing physical protection and an optimal moisture environment and accelerate the healing process by promoting cell proliferation and also by preventing infection (Martin et al., 2013).

Chitosan (Ch) is produced commercially by deacetylation of chitin and it is the main element of exoskeleton of crustaceans e.g. shrimp and crabs. Due to its natural biological origin, chitosan is a very good material for cell adhesion and proliferation. It is natural polysaccharide similar to glycosaminoglycan gives property to stimulate growth factor. It has unique features such as natural antibacterial, water retention capacity and biocompatible, biodegradable in nature (Croisier and Jérôme, 2013; Nguyen et al., 2013). Ciprofloxacin is a well-known broad spectrum antibiotic intended for topical use (Sinha et al., 2011). It has been chosen based on different mechanism of action from the other category of antibiotics e.g. penicillin. Currently, Ciprofloxacin is available in the market as a cream or ointment that must be applied three times daily which leads to the patient in-compliance. So controlled delivery of ciprofloxacin from suitable hydroactive biomaterials may be a possible solution to the challenges related to wound infection and patient compliance. The main objective of the present study is to

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prepare biocompatible films using chitosan for an effective drug delivery system. The three different chitosan films namely Ch1 (1%) Ch1.5 (1.5%) and Ch2 (2%) were prepared and were loaded with ciprofloxacin drug, which was further characterized using different physico-chemical parameters. The effective biofilm concentration impregnated with ciprofloxacin was also determined, chitosan biofilm without ciprofloxacin served as a control.

2. Materials & methods

2.1. Materials

Chitosan (degree of deacetylation ≥ 75%) and ciprofloxacin were procured from Hi-media chemicals Pvt. Ltd., Mumbai, India. Tissue culture media and antibiotics were purchased from Sigma-Aldrich, India. NIH 3T3 fibroblast cell line was obtained from the National Centre for Cell Science (NCCS), Pune, India. Bacterial strain *S. aureus* (ATCC 11632), *E. coli* (ATCC 10536) and *P. aeruginosa* (ATCC 10145) were obtained from Institute of Microbial Technology, Chandigarh, India. Distill water and miliQ water were used throughout the experiments.

2.2. Fabrication of chitosan film

The chitosan films were prepared at different concentrations like 1%, 1.5% and 2% (w/v) mixed with 2% (v/v) acetic acid by solvent casting method (Fig. 1). The chitosan with acetic acid mixture was dissolved using sterile distilled water and was stirred overnight to ensure complete solubility. Glycerol (300 µl) was added to each solution as a plasticizer, mixed and the solution was poured into a petri plate. Samples were incubated at room temperature (37 °C) until dried and stored in a desiccator. Further, the drug (D) Ciprofloxacin was loaded on various concentrations of chitosan biofilms, described in Table 1. The drug was impregnated in the film to prevent and eradicated the microorganism present in the wound site that ultimate reduce the inflammatory cells (Boateng et al., 2008).

2.3. Swelling study

Chitosan film of various concentrations of size $2 \times 2\,cm^2$ was immersed into phosphate buffer saline (PBS, 7.4 pH, 37 °C) for 24 h to determine the water uptake property. At specific time interval, dry and wet weight of film was measured and % swelling was calculated by the following formula.

$$\% \text{ Swelling} = (Ww - Wd)/Wd \times 100$$

where W_d and W_w is the dry and wet weight of the film (Fan et al., 2016).

Table 1
Formulations.

Film Code	Chitosan Concentration (%)
Ch1	1
ChD1	1 + Drug
Ch1.5	1.5
ChD1.5	1.5 + Drug
Ch2	2
ChD2	2 + Drug

2.4. Mechanical properties

The thickness of chitosan film was determined by Mitutoyo digital screw gauge. Samples were analyzed for its thickness in six different places and recorded. The mechanical property of the film was measured by using the Universal testing machine (INSTRON model 1405). A dumb-bell shape of films size $5 \times 1\,cm^2$ was fixed in the Universal testing machine and % of the elongation at break was measured as described in ASTM D638 & D882 (ASTM, 1994)[6] (Lopez-Mata et al., 2013).

2.5. Fourier transform infrared (FTIR) analysis

Chitosan (Ch) and Ciprofloxacin drug (D) were subject to the FTIR analysis. The samples were made a pellet using pure KBr in the ratio of 10:90 and fixed in the sample holder. An infrared spectrum was recorded from 400 to $4000\,cm^{-1}$ with a resolution of $5\,cm^{-1}$. Ciprofloxacin drug (ChD) loaded chitosan film undergone Attenuated Total Reflection using FTIR-ABB 3000 spectrometer, Canada (Chhavi et al., 2012).

2.6. Scanning electron microscopy (SEM) analysis

The surface morphological characterization of chitosan film was analyzed by Scanning Electron Microscopy (Phenom Pro). The film sample was placed over the bronze stub containing a double sided tape and coated with gold before evaluation (Balaji et al., 2012).

2.7. In-vitro drug release

Franz-type diffusion cell containing phosphate buffer saline (PBS, 7.4 pH, 37 °C) was used for *in-vitro* drug release for 7 days. To determine release behavior at a specific time intervals (1,2,3,4,6,8,10,12,24,30,36,48,72,96,120,144,168 h) aliquot of 1 ml was taken and measured by UV–vis spectrophotometer at 276 nm and replenished back with fresh PBS (Shanmugasundaram et al., 2005).

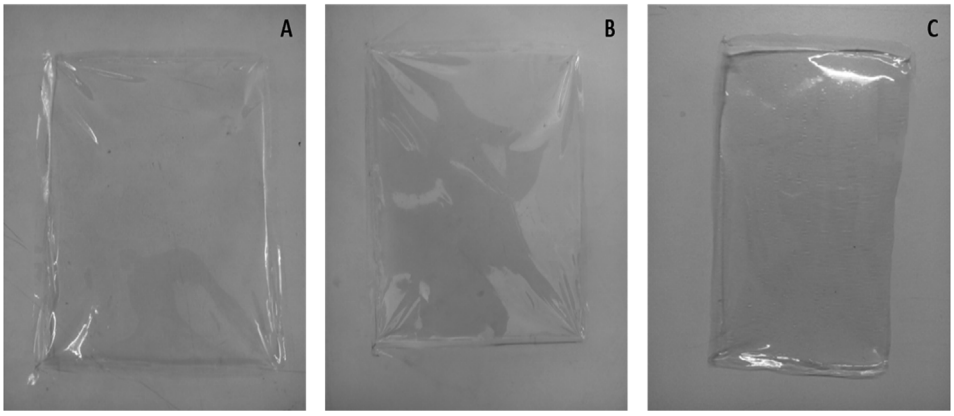


Fig. 1. Images of chitosan film from different concentration (A) 1% (B) 1.5% (C) 2%.

2.8. In-vitro biocompatibility test

To determine the biocompatibility of formulated chitosan film, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) test was performed using NIH 3T3 Cell lines. 3T3 fibroblast cell line was obtained from the National Centre for Cell Science (NCCS), Pune, India. DMEM medium supplemented with antibiotic was used for culturing. The cell line has grown over the films at a density of 5×10^4 cells/well and incubated for 37 °C in humidified atmosphere of 5% CO_2 for the period of 24 h, 48 h, 72 h, 4 days and 5 days. After specified time interval 100 µl of MTT solution was added to each well and incubated for 4 h for 37 °C in humidified atmosphere of 5% CO_2. The medium was aspirated and 500 µl of DMSO (dimethylsulfoxide) was added to each well and shake for 10 min to dissolve the formed formazan crystal. The absorbance was measured at 570 nm using Universal microplate reader. All experiments were done in triplicate and shown in mean ± S.D. value to ensure the reproducibility of the results (Giriprasath et al., 2015).

2.9. Antibacterial activity

To examine the antibacterial activity of the films gram-positive bacteria *S. aureus* and gram-negative *E. coli* and *P. aeruginosa* was used. 5×10^5 cfu/ml of bacterial inoculum was prepared and inoculated in the agar plate. Samples of size 1×1 cm^2 was placed over the agar plate and incubated for 24 h at 37 °C. After 24 h zone of inhibition was measured and recorded (Valderama et al., 2015).

3. Results & discussion

3.1. Swelling study

The swelling behavior of the chitosan films was analyzed at a different time interval, which shows the initial first hour swelling was 248 ± 4.53, 154 ± 5.1 and 218 ± 6.1% for Ch1, Ch1.5 and Ch2 respectively, which is good for wound dressing material. The Ch1 showed initially three hour high swelling compare to other film but Ch1.5 and Ch2 consistently increasing the swelling behavior. Overall, the Ch2 film has the highest swelling of 290 ± 10% on 24 h (Fig. 2). Swelling study was performed to understand the absorbing capacity of individual films, which is an important parameter for wound dressing material. Moreover, the wound dressing material has to absorb the excess wound exudate and keep the wound surface dry and maintain the optimum moisture level. Swelling represent the capacity of film to absorb exudate (Nguyen et al., 2013).

3.2. Mechanical properties

The mechanical property of the chitosan film with thickness was analyzed and presented in Table 2. The films were in the range of 50 µm–130 µm and uniform throughout the film. The thinnest film was 1% concentration of chitosan (Ch1) and the thickness of the film increases with the concentration. The addition of drug ciprofloxacin has no significant difference in the thickness of the film. The mechanical strength of the film was found to be 43, 32 and 38 (Mpa) for the Ch1, Ch1.5, and Ch2 respectively. Good mechanical property of the film is important criteria for any biomaterial, which support the tissue during development (Srinivasa et al., 2007; Sezer et al., 2007).

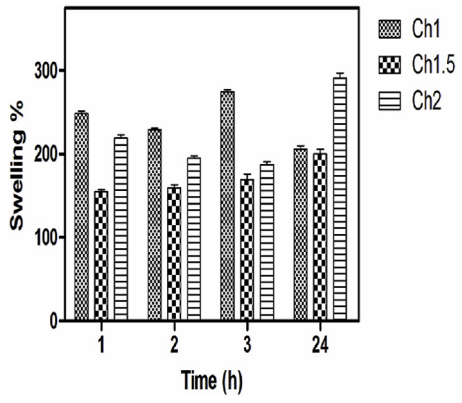


Fig. 2. Swelling study of the chitosan film.

Table 2
Mechanical property of the chitosan films.

Formulation	Thickness (µm)	Tensile Strength (MPa)	Elongation at break (%)
Ch1	50 ± 0.18	43 ± 0.2	5 ± 0.2
Ch1.5	90 ± 0.2	32 ± 0.3	4.5 ± 0.12
Ch2	130 ± 0.3	38 ± 1.5	7 ± 0.5

Results are presented as mean ± SD (n = 3).

3.3. Fourier transform infrared (FTIR) analysis

The FTIR spectra for chitosan, ciprofloxacin and ciprofloxacin loaded chitosan film are presented in Fig. 3. The chitosan has broad band at 3250 cm^{-1} due to $-NH_2$ stretching vibration with $-OH$ hydrogen bond. The two bands observed at 2920 and 2876 cm^{-1} is characteristic for $-CH_2$ and CH tertiary. Ammonium side chain of chitosan was observed band at 1630 and 1515 cm^{-1}. The vibration of $-CH_3$ of amide group was observed at 1375 cm^{-1}, C—C and C—O stretching band was observed at 1090, 1065 and 1120 cm^{-1}. In ciprofloxacin spectra showed carbonyl stretching at 1722 cm^{-1} and stretching vibration of C—F at 1290 cm^{-1}. The band at 3043 cm^{-1} and 2918 cm^{-1} is the C—H stretching vibration of phenyl frame of ciprofloxacin. The FTIR of composite film exhibit characteristic absorption of both chitosan and ciprofloxacin with slight shift showed the compatibility of the drug with the polymer (Sinha et al., 2011; Kloster et al., 2015).

3.4. Scanning electron microscopy (SEM) analysis

Scanning electron microscopy analysis showed the morphology microstructure of different concentration of chitosan film Ch1, Ch1.5,

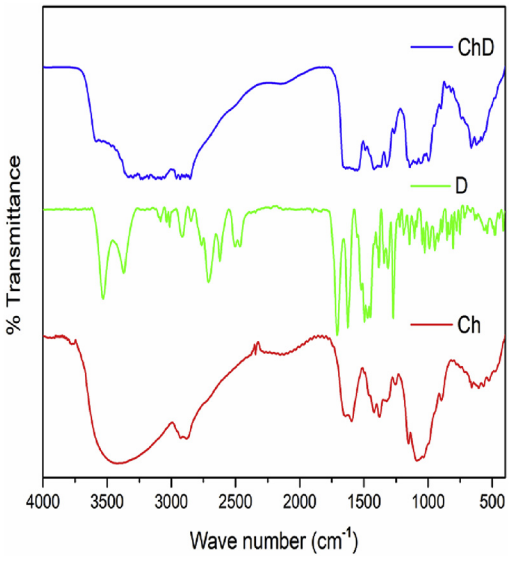


Fig. 3. FTIR spectrum of chitosan (Ch), ciprofloxacin (D) and ATR-FTIR of chitosan-ciprofloxacin (ChD) film.

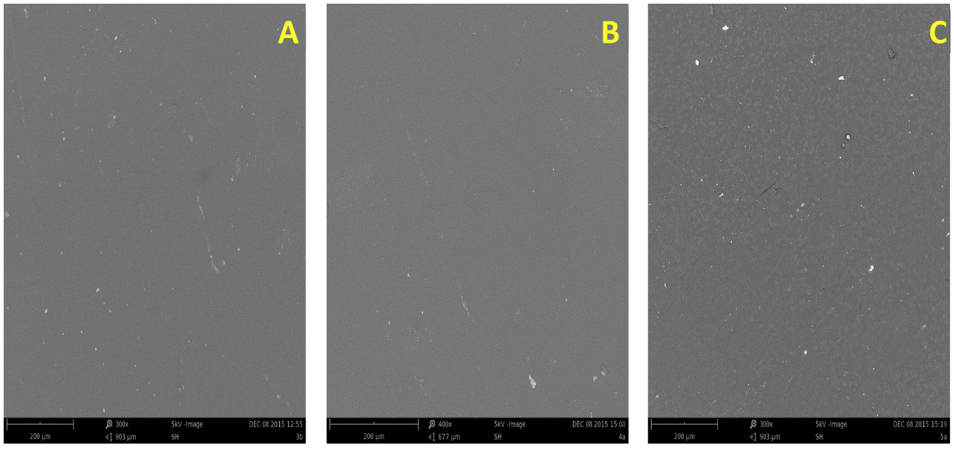


Fig. 4. SEM images of chitosan film from different concentration (A) 1% (B) 1.5% (C) 2%.

and Ch2 and depicted in Fig. 4. All the film show the plain surface with good texture and porous nature. While increasing the concentration of chitosan the porous nature increased. The porous nature of film absorbs excess wound exudates, makes wound dry and help to maintain optimum moisture level. Which helps to heal faster and free from bacterial infection (Pandima Devi et al., 2012).

3.5. In-vitro drug release

The *in-vitro* ciprofloxacin drug release study was conducted in PBS and presented in Fig. 5. The initial drug release was observed at 2 h intervals, which shows the 13, 19 and 21% of drug release respectively for Ch1, Ch1.5 and Ch2. This type of initial burst release is good for decrease the load of microbes present in the wounds. The sustained release of ciprofloxacin drug was 65, 84 and 96% respectively for Ch1, Ch1.5 and Ch2 for the duration of 48 h. The release of ciprofloxacin drug was sustained and increased with increasing concentration of the chitosan in the prepared film, probability due to swelling property and loading of the drug (Yang et al., 2010; Martínez et al., 2015). The release of drug is lesser in Ch1 and Ch1.5 may due to less polymer concentration comparatively (Sinha et al., 2011).

3.6. In-vitro biocompatibility assay

The *in-vitro* biocompatibility for the chitosan biofilms was evaluated using NIH 3T3 cell lines for 5 days and shown in Fig. 6. The percentage of cell viability using MTT test shows that the formulated biofilm is biocompatible, metabolically active and well distributed. Over the days

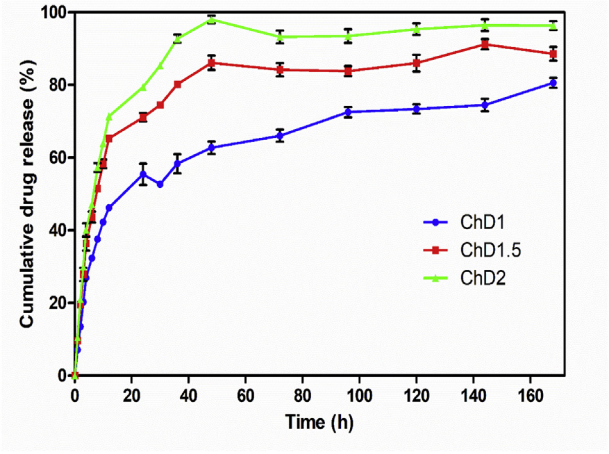


Fig. 5. *In-vitro* drug release profile.

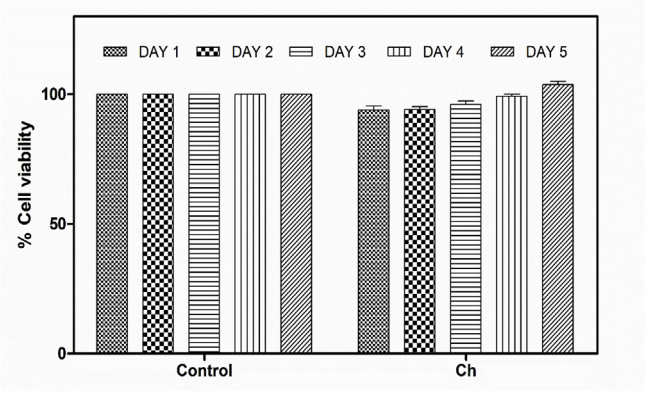


Fig. 6. MTT viability assay of chitosan film for 1, 2, 3, 4 and 5 days.

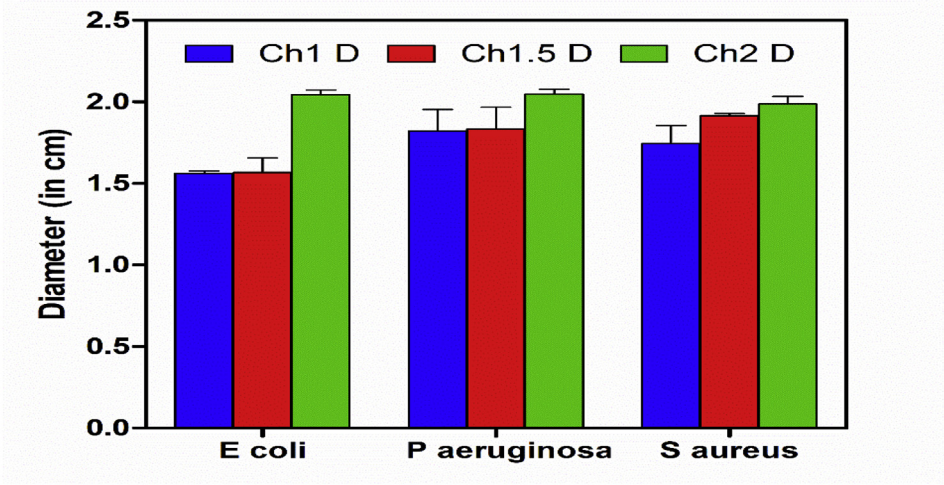
cell density and growth was increased for the formulated film. The duration of 5 days has been chosen to see the biocompatibility of film and fabricated biofilms do not pose any cytotoxic and adverse effect in NIH 3T3, which is a prerequisite for biological application in tissue engineering (Balaji et al., 2012; Yang et al., 2010; Guzman et al., 2014).

3.7. Antibacterial activity

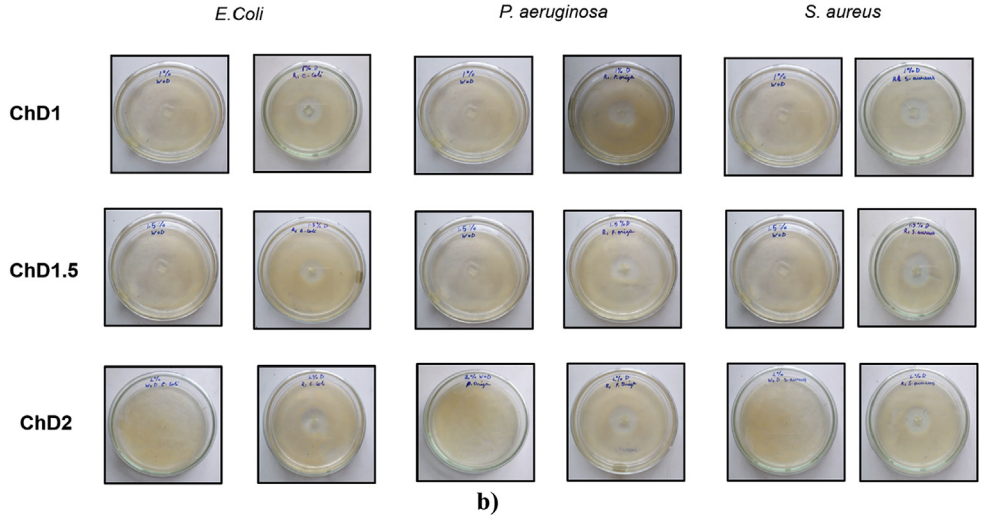
Antibacterial activity of chitosan biofilms namely Ch1, Ch1.5 and Ch2 loaded ciprofloxacin drug was evaluated against three different bacteria such as *E. coli, P. aeruginos* (gram negative) and *S. aureus* (gram positive). *E. coli, P. aeruginos, S. aureus* is most common causative microorganisms present in the wound site (Bowler et al., 2001). All the films exhibit the antibacterial activity against three different bacteria with zone of inhibition shown in the Fig. 7. Samples without ciprofloxacin drug does not exhibit any zone of inhibition. The results indicate the Ch2 film exhibit a higher zone of inhibition compare to the other film. It may be due to the sustained release of the drug as revealed in the *in vitro* drug release profile (Rathore et al., 2016).

4. Conclusion

Gupta et al. (2019) reported that chitosan has promoting wound healing and early osteogenesis in tooth. Three different concentrations of chitosan films impregnated with ciprofloxacin were successfully prepared and characterized by different parameters. The increase in chitosan concentration in film increases thickness, *In-vitro* drug release as well as antibacterial activity except 1% of chitosan containing drug had more water uptake initially compare to others. All formulation has good texture and biocompatibility. The results imply that the



a).



b)

Fig. 7. Antibacterial activity of the chitosan film.

ciprofloxacin loaded chitosan film may have potential for wound healing applications.

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