Porous Chitosan Scaffold for Sustained Release of Ciprofloxacin Hydrochloride as a Potential Wound Dressing

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Abstract

Scaffolds or extracellular matrixes, made by polymers, are devices that can be utilized in wound healing as framework which promote cellular proliferation and growth as well as delivery system for controlled release of antibiotics to hasten the healing process by preventing microbial infections. To perform these functions, chitosan was chosen as scaffolding material due to its biocompatibility, biodegradability, non-toxicity, ease of availability, non-immunogenic and anti-bacterial properties. Scaffold of chitosan was made by optimized method of freeze gelation. The broad spectrum antibiotic ciprofloxacin was incorporated and its sustained release from the scaffolds, without the loss of scaffold structure and bioactivity, was done. Experiments were carried out at different pH 4.5, 5.5, 6.5 and 7.4 as it is known that the skin pH of the infected area of the wound get decreased which depends on the extent of bacterial infection. In all the tested scaffolds, burst effect for drug release was observed, 15 % of drug got released within 1 hr, then after slow release was observed till 7 days which support in maintaining the drug concentration in the therapeutic range at the infected site. Degradability of the scaffold was also studied to find out the duration till it supports the healing tissues at the wound site. Simultaneously, bacterial growth evaluation was done on *Stapylococcus aureus* and *E.coli* culture, typically found in skin, nasal passages and mucus that can cause wide range of infections. It was clearly seen that the released drug was effective for suppressing the growth of both the microorganisms at the specified concentration. Moreover, the released drug concentration was sufficient to inhibit bacterial growth at the wound site that hastens the healing process.

Keywords - Polymeric Scaffold, Chitosan, Biodegradable Polymer, Controlled Release System, Ciprofloxacin Hydrochloride, Wound Healing.

Introduction

Biodegradable polymers have diverse application in medical devices as implants, sutures, orthopaedic repair material and controlled release of a broad range of pharmaceuticals (Persidis, 1999; Kanawy *et al.*, 2002). These polymers have become much popular in design of various drug delivery systems as they degrade in the body to small molecular weight compounds that are either metabolised or excreted. This behaviour gave the answer for removal of the carrier after the device is exhausted (Ramchandani and Robinson, 1998). The management and treatment of external wounds and of internal traumas which are

consequences of surgery are areas of intense research and commercial interest.

Moreover, these are areas in which recent development has significantly improved the quality of life of patients (Sharon *et al.*, 2006). These dressings and compositions can provide drug delivery features having sustained release of pharmaceuticals. The dressing can be utilized in the form of scaffold whose material can be formed from bioabsorbable polymers such as (but not limited to) polymers of lactic and glycolic acids, copolymers of lactic and glycolic acids, poly (ether-co-esters), poly (hydroxybutyrate), copolymers of lactic acid and aminocapronic acid, lactide polymers, copolymers of poly (hydroxybutyrate) and 3 hydroxyvalerate, polyesters of succinic acid, poly (N-acetyl-D-glucosamine), cross-linked hyaluronic acid and cross-linked collagen. The bioabsorbable scaffold material that is useful in the present invention can dissolve in exudates at rate equal to, or slightly slower than the rate of wound healing. The rates of bioabsorption of the scaffold material can be tailored, if desired, according to the expected time of healing of the wound to which it is to be applied. For example, a scaffold material that is bio-absorbed within one or two weeks may be particularly useful for a rapidly healing wound, while a scaffold that is bio-absorbed within approximately 1-2 months can be used for chronic wounds and wounds that require longer healing times. The scaffold can also be impregnated with other bioactive agents such as drugs, vitamins, growth factors, therapeutic peptides.

Chitosan (Cht), the deacetylated derivative of chitin, has special interest in the biomedical industry because of its excellent biodegradability, biocompatibility, antimicrobial and accelerated wound healing properties (Hirano *et al.*, 1994; Malette *et al.*, 1983; Qurashi *et al.*, 1992; Wel *et al.*, 1992). It has good gelatinizing and film forming properties. When it is dissolved in dilute acetic acid solution, the amino groups become protonated and associated with acetate counter-ions, making the charged polymer soluble. Therefore, net negatively charged compounds such as DNA, glycosaminoglycans, and most proteins can be incorporated into chitosan without the use of harsh and denaturing organic solvents, such as methylene chloride, which are needed for film preparation of many biodegradable polymers. Therefore, chitosan has been investigated extensively in the pharmaceutical industry for its potential use in the development of controlled release implant systems (Wang *et al.*, 2005). Moreover, chitosan is easily hydrolyzed and metabolized by various chitosanases and lysozyme, hence considered biodegradable. The biodegradation leads to the release of aminosugars which can be incorporated into glycosaminoglycans and glycoproteins metabolic pathways and excreted (Chatelet *et al.*, 2001).

Ciprofloxacin (CFX) has been the most widely used fluoroquinolone for treatment of bacterial infection. The minimal inhibitory concentration (MIC) of CFX is low (0.25–2 μg/ml) for most of the pathogens that hamper normal wound healing such as *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* (Grady, 1992; Armstrong and Elsberry, 1997). Ciprofloxacin is a second generation fluroquinolone derivative, exhibiting activity against a wide range of Gram-negative and Gram-positive facultative bacteria.

During wound the tissue pH get decreased from the normal value (Lin and Lin, 1992). The aim of this research was to study the effect of pH on CFX release from the chitosan scaffold and evaluation of the effect of released CFX on the normal wound pathogen. The polymeric scaffold was prepared by freeze gelation method (Shu, *et al.*, 2001) and CFX was incorporated into the scaffold at specified concentration. The polymer drug interaction was studied using FTIR and morphological details were

described by Scanning Electron Microscopy. Simultaneously, Degradability of the scaffold was done in PBS (Phosphate Buffer Saline) to show the duration till it is available at the wound site.

Materials and method

Materials

Chitosan was purchased from Marine Chemicals, Cochin, India with 80% degree of deacetylation. Ciprofloxacin hydrochloride was a kind gift from Elcon Drug and Formulation Ltd., Jaipur, India. *E.Coli* MTCC 739 and *Staphylococsccus aureus* MTCC 2940 were procured from Microbial Type Culture Collection (MTCC), India. Other reagents were all analytical grade.

Preparation of drug-loaded chitosan scaffold

Chitosan (Cht) scaffolds were prepared by freeze gelation method (Hirano *et al.*, 1994). Chitosan solutions with concentrations of 1 to 3 wt% were prepared by dissolving in 0.2 M acetic acid. The solution was stirred at 100 rpm for 2 h to obtain a homogeneous polymeric solution and sonicated for 10 min to remove air bubbles trapped in the viscous liquid. It was poured into the petridish having 4cm diameter and rapidly transferred into a deep freezer at a preset temperature (−20°C) in deep freezer (URC-V) to solidify the solvent and induce solid-liquid phase separation. The solidified mixture was maintained at the temperature for 12 h. The solidified sample was gelatinized by adding 2 M NaOH. The scaffolds formed were washed with 90% alcohol to remove any residual solvent. The above scaffolds were lyophilized (Decibel Digita Technology, DB-31533) to get dried sample. These dried scaffolds, with an average thickness of 2mm determined by thickness instrument Micrometer MI, Cheminstruments, were cut into circle of diameter 4cm. The chitosan scaffolds were designated as Cht-1, Cht-2, Cht-3, Cht-4 and Cht-5 (chitosan contents having 1, 1.5, 2.0, 2.5 and 3.0 wt % respectively). Following the above method, specified amount of ciprofloxacin hydrochloride (0.5 mg/ml) was incorporated into the polymeric solution (1.5, 2.0 and 2.5 wt %) before sonication producing drug loading scaffolds designated as CP-1, CP-2 and CP-3 having above polymeric solutions, respectively. Drug loading was the difference between total drug added into the solution and drug concentration in washing solution which was calculated by taking absorbance at 277nm using spectrophotometer (Shimadzu, 1700 UV).

Drug assay

Ciprofloxacin was assayed by UV-Visible spectrophotometric method. Drug was solubilised in water and a range of concentrations 10-100µg/ml was prepared. Standard curve was obtained by taking absorbance of the standards at 277nm using spectrophotometer (Shimadzu, 1700).

Release study

Release of ciprofloxacin from the scaffold was assayed in triplicate under sink conditions. The polymeric scaffold is placed in opaque flasks with citrate buffer of pH 4.5, acetate buffer of pH 5.5, phosphate buffer of pH 6.5 and 7.4 isotonic solutions having 0.02% w/v sodium azide, at 37 °C. At suitable time intervals, a part of the total volume of the aqueous solution was withdrawn and replaced immediately with fresh buffer. After filtration, the amount of ciprofloxacin was determined at 277nm using spectrophotometric method.

FT-IR analysis

The FT-IR spectra of pure chitosan, ciprofloxacin hydrochloride and polymeric scaffold with the antibiotic were recorded within KBr pellets on a FTIR spectrometer, Shimadzu, 8400S.

Bacterial evaluation

Effectiveness of the released drug was studied, which involved the use of bacterial colony formation and zone of inhibition. In this test, scaffolds sections (1 X 1 cm2) of varying formulations (i.e., Chitosan with and without drug) were incubated at 37 °C for 4 h on agar plates, allowing the incorporated drugs to diffuse from the scaffold into the agar (in the case of medicated scaffolds). Each scaffold section contained between 0.5 and 0.6 mg of CFX. Upon the removal of scaffold the plate was allowed to dry for 2 h at 37 °C. A 100 µl aliquot of *S. aureus* and *E. coli* were spread directly onto the plate, and the plate was incubated overnight at 37 °C. Bacterial growth was visualized directly on the plate.

Morphology observations

The surface morphologies of the Chiotsan (Cht) and Cht -CFX scaffolds were examined using scanning electron microscopy (SEM) Hitachi S-570 (Japan). Cross-sectional samples were prepared by fracturing scaffold in liquid nitrogen. Prior to observation, samples were arranged on metal grids, using double-sided adhesive tape, and coated with gold under vacuum before observation.

Results and Discussion

Selection of polymeric concentration for scaffold preparation

The scaffolds prepared by using different polymeric solutions having concentrations 0.5, 1, 1.5, 2, 2.5 and 3 wt% were studied. Scaffolds of 0.5 and 1 wt% polymeric solution were very weak and easily breakable whereas 3 wt% polymeric solution was very viscous and not pourable. Hence, 1.5, 2.0 and 2.5 wt % concentrations were taken for further studies.

Release study

Effect of polymeric concentration into the matrix

Release of drug from the scaffold was reported from three different concentrations of polymeric solutions, viz., 1.5, 2 and 2.5 wt% which were kept in phosphate buffer of pH 7.4. It is very clear from the Fig. 1 that release is better from 2 wt% polymeric solution scaffold i.e. CP-2. Above and below the concentration the release was at lower side. Although, the drug loading increases from 2, 5 and 9 % in CP-1, CP-2 and CP-3 respectively but the drug release was not increasing accordingly. It can be inferred that drug loading may be less in 1.5 wt% polymeric solution due to low concentration of polymer. Whereas at 2.5 wt% concentration drug loading is better but drug may get entrapped strongly which resulted into its comparatively lesser concentration into the release media.

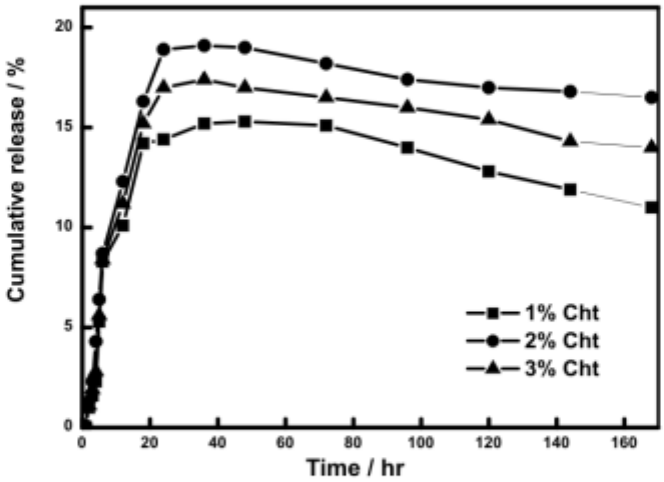

Figure 1: Effect of chitosan concentration on release of CFX; CP-1, Chitosan 1.5 wt%, CP-2, chitosan with 2 wt%, CP-3 chitosan with 2.5 wt% all having ciprofloxacin 0.5 mg/ml

Effect of pH

The drug release from loaded scaffold CP-2 in four different buffer solutions citrate buffer of pH 4.5, acetate buffer of pH 5.5, phosphate buffer of pH 6.5 and 7.4 (0.2% sodium azide in the buffered solution to prevent the fungal activity). From Fig. 2, it was inferred that the release was very sensitive to the pH of the medium. The release was accelerated with decrease of pH, because the electrostatic interaction between anions and chitosan was greatly influenced by solution pH (Shu *et al.*, 2001). The decrease of pH weakened salt bonds and therefore, facilitated scaffold swelling, thereby accelerating drug release. The pH also has a slight effect on the solubility of ciprofloxacin hydrochloride. A lower pH leads to a better solubility of ciprofloxacin hydrochloride, which results in higher drug release rate. But compared to the strong influence of pH on the scaffold, pH effects on ciprofloxacin hydrochloride could be neglected.

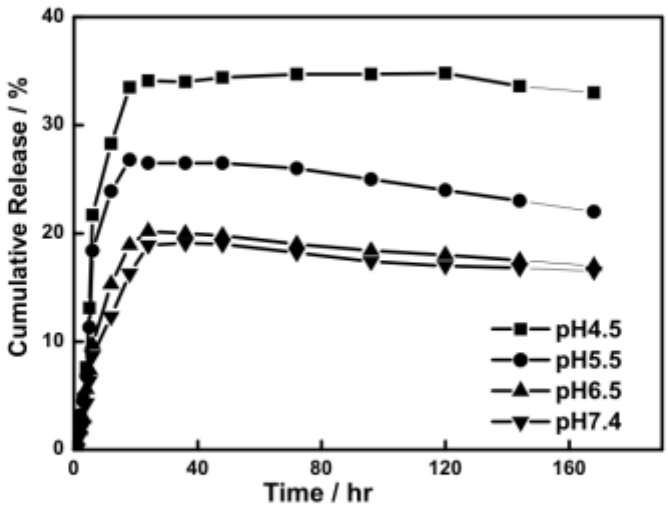

Figure 2: Effect of different medium pH on CFX release.

FT-IR analysis

The FT-IR spectra of chitosan, ciprofloxacin hydrochloride and drug loaded scaffold. In Fig. 3 two characteristic absorption bands in pure chitosan at 1660 cm^{-1} and 1595 cm^{-1} were detected and attributed to amide I (C=O) and amide II (N–H), respectively; 1379 cm^{-1} was attributed to the distorting vibration of C–CH_3 (Sannan *et al.*, 1978). The characteristic absorption bands at 1290 and 1622 cm^{-1} of ciprofloxacin hydrochloride were due to the stretching vibration of C–F bond and the vibration of phenyl framework conjugated to –COOH, respectively; the stretching vibration at 1711 cm^{-1} was due to –COOH and, at 3043 cm^{-1} and 2918 cm^{-1} were observed the stretching vibrations of C–H from the phenyl framework. Through the FT-IR spectra of Chitosan scaffold, it can be seen that the characteristic absorption bands at 1660 cm^{-1} and 1595 cm^{-1} of CP shifted to lower wave number at 1647 and 1550 cm^{-1}, respectively; and also that the characteristic absorption band at 3419 cm^{-1} had shifted to a lower wave number at 3408 cm^{-1}. All these results indicated that the drug used in this work had strong hydrogen bonds and ionic bonds with the polymeric matrix of the scaffold. At the same time, there were no new characteristic absorption bands of drug loaded scaffold, permitting conclusion that there were no obvious chemical reaction between the drug and the matrix. As an important result, ciprofloxacin hydrochloride did not lose its activity in the drug-loaded scaffold and hence equally effective after getting released from the scaffold.

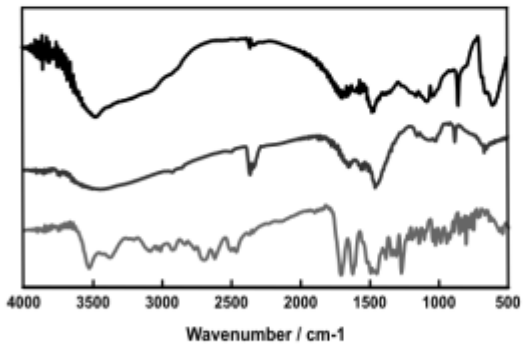

Figure 3: FT-IR of (a) Ciprofloxacin, (b) Chitosan, and (c) polymeric scaffold with the drug, Ciprofloxacin

Scanning electron microscopy

Analysis of the morphologies of Chitosan and Chitosan with CFX scaffold was done by Scanning Electron Microscopy (SEM). Fig.4 shows that the surface is microporous and drug incorporation did not change the surface for any considerable extent. Again, the result obtained here indicates good compatibility between the matrix and the drug, ciprofloxacin hydrochloride.

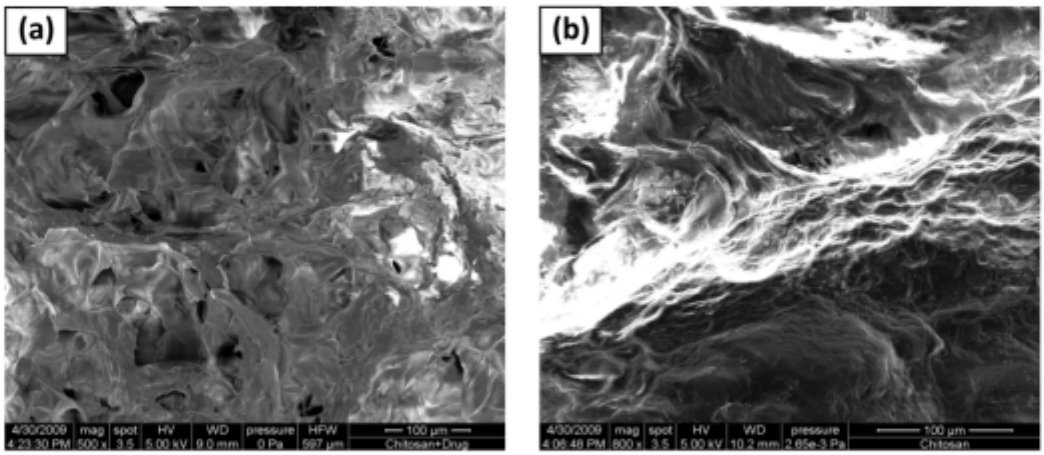

Figure 4: Scanning electron microscopy of chitosan scaffold (a) with and (b) without drug

Bacterial evaluation

The effectiveness of the released ciprofloxacin hydrochloride was investigated using gram positive *Stapylococcus aureus* (Lowy, 1998) and gram negative *E.coli*, which are commonly present at the wound site. The experiment showed effectiveness of the drug against wider range of microoraganisms. The liquid bacterial culture test, the efficacy of the medicated scaffolds using a static system for bacterial growth on agar plates was examined. The growth of *S. aureus* and *E. coli* was visualized directly on the plate to assess the viability of the medicated scaffold (Fig. 5). Agar regions, where the medicated scaffold was placed, clearly show inhibition of bacterial growth after 24h of incubation at 37°C. Additionally, the released drugs from the medicated scaffold (1x1 cm) can also inhibit bacterial growth in a much larger area than the scaffold size due to the diffusion of the drug onto the agar. In contrast, scaffolds containing no drug exhibited no inhibitory effect and bacteria grew robustly. It is interesting to note that as the drug potency decreases at longer times (48 h at 37 °C), bacterial cells begin to proliferate. Despite even at these longer time points, there was still sufficient bioactive released drug to substantially inhibit bacterial growth.

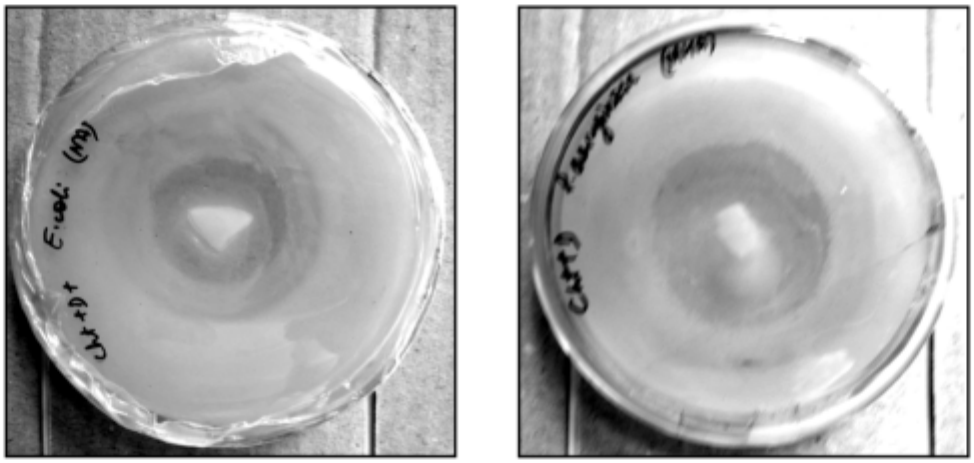

Figure 5: Zone of inhibition of *E. coli* and *S. aureus* seen after incubating them on plates for 24 hr at 37°C where previously scaffolds with drug were kept

Table 1: Zone of inhibition due to releasd drug from scaffolds made by chitosan or chitosan mixed with other polymer

Sl.No	Sample	Microorganism	Average zone of inhibition (cm)
1.	Chitosan with drug	*E. coli*	3
2.	Chitosan with drug	*Pseudomonas aeruginosa*	3.7

Conclusion

Drug loaded scaffold of chitosan polymer was made by freeze gelation method. Ciprofloxacin was used as a model drug to show the effectiveness of these scaffolds in wound healing. The chemical and morphological characterization showed that there was a good compatibility between the matrix and the drug used due to hydrogen bond interaction; hence the drug was still active after getting released from the

matrix. The results of controlled release tests showed that the amount of ciprofloxacin hydrochloride released was optimum at 2 wt% of polymeric concentration. The scaffold was also pH sensitive due to chitosan and drug, both solubility get changed by changing pH. It was seen that drug loading was very low but as the MIC is very less for the antibiotic it was not a problem. Moreover, the zone of inhibition signifies that the released drug was quite effective in inhibiting the microorganisms which are commonly found at the infected site. The strength of the scaffold which was started getting solubilised supports the idea that the scaffold would be effective in treating acute wounds. As the polymer is biodegradable, the property is additive in release profile of drug as the initial release is due to diffusion but later by degradation of the polymeric structure.

The results indicated that the scaffolds had porous structure with occasionally interconnected pores. It is worth mentioning that the interconnected pore structure is one of the essential characteristics of a perfect scaffold because it allows the tissue ingrowth as well as blood and nutrient supplies for cells to be alive. It can be concluded that the polymeric scaffold is useful in drug delivery and it can be used to enhance the wound healing by prolonged release of drug to the infected site.

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