International Journal of Drug Delivery 2 (2010) 173-182

http://www.arjournals.org/ijdd.html

Research article

ISSN: 0975-0215

Preparation and evaluation of ciprofloxacin loaded chitosan-gelatin composite films for wound healing activity

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Abstract

Back ground: Natural polymers are used as lead compounds for design of drugs in treatment of different ailments. Chitosan and gelatin have proven wound healing properties individually. As both have wound healing property, the combination of these two polymers and incorporation of drugs into the composite films may show improvement in wound healing activity. Thus, the composite films and drug loaded films were evaluated for various *in vitro* evaluation tests to ascertain the applicability of prepared combination for wound healing activity. The composite films were prepared with increase in gelatin concentration and the drug loaded films were prepared with increased concentrations of drug in optimized composite film. These films were evaluated for thickness, folding endurance, water absorption capacity, antibacterial activity, tensile strength, drug load, content uniformity, *in vitro* drug release by diffusion studies and *in vivo* wound healing studies by excision wound model using albino rats. **Results**: The drug loaded films shown significant difference in folding endurance, water absorption capacity, antibacterial activity when compared to optimized blank composite film. There was no significant difference in thickness and tensile strength of drug loaded films when compared to blank composite films. Percentage of wound contraction was more for wounds treated with ciprofloxacin loaded composite film than blank composite film. **Conclusions**:With the above results, it was concluded that ciprofloxacin loaded chitosan-gelatin composite films had shown more wound healing property than chitosan-gelatin blank composite film and blank chitosan film without interfering in strength of film.

Keywords: Ciprofloxacin; Chitosan; Gelatin; Drug loaded films; Wound healing; Tensile strength.

Introduction

Wound healing is the body's natural process of regenerating dermal and epidermal tissue. It is the process where by the body restores the injured part to as near its normal condition as possible. Though wound healing takes place naturally on its own, some of complications like sepsis, disruption of tissue and skin layer, maggot's formation, extension of infection to adjacent and interior organs occur in major cases. To prevent extensive loss and damage to the tissue, skin grafting [1] and biological dressings [2] were

doi:10.5138/ijdd.2010.0975.0215.02027

developed. The ability of the skin to repair itself after a minor wound is remarkable, but when the damage is severe or occurs in large amounts of skin area, proper and immediate coverage of wound surface with an adequate dressing is needed to protect the wound and to accelerate wound healing. Ultimately the immediate wound coverage, temporary or permanent, is one of the principal goals of wound management. For this films made with biomaterials are becoming popular due to many advantages.

Biomaterials are natural polymers and are biodegradable. These are used in regenerative medicine, implantable materials, controlled release carriers or scaffolds for tissue engineering. Cellulose, chitin, chitosan and gelatin are widely used natural polymers. Natural polymers when used as drug delivery carriers, they are degraded into biologically accepted compounds, often through the process of hydrolysis, which leave the incorporated medications behind [3]. The major advantages of natural polymers are good cytocompatibility, biodegradable and do not require any surgery for removal of polymers [4].

Biological dressings like fibrin glue [5], gelatin sheets, chitosan films [6], collagen [7] are popular for quicker wound healing. These polymers when used in combination such as fibrin-gelatin, fibrin-chitosan shown better results, than when used alone.

Chitosan is nontoxic, biocompatible [8-10], biodegradable polymer [4,6,9-11]. It is used in drug delivery, cell delivery systems, orthopaedics, wound healing[6], ophthalmology, and bone healing [12]. It enhances the function of polymorphonuclear cells, macrophages [13] and fibroblastic proliferation and migration [14]. It exhibits antimicrobial activity against bacteria [15], fungi, and yeast. It is hypoallergenic, has rapid blood clotting property, haemostatic and acts as fat attractor by binding to dietary lipids [16].

Gelatin is also a natural polymer derived from collagen of animal skin and bones. It is translucent, colorless, brittle and tasteless. It is biodegradable in nature. It has good film forming property and known for its wound healing properties by preventing fluid loss due to exudation [17]. It is a good source of protein and promotes general joint health and stiffness in athletes.

Impaired wound healing due to infections and other above mentioned complications spurred the search for drug loaded films [18]. The drug loaded films are prepared by incorporating drugs like antibacterials, antibiotics in the films. The drug loaded films act as barrier to micro-organisms when applied on to wound and thus prevent secondary infections to augment the process of wound healing by stimulating wound healing environment. Therefore drug loaded films are more useful to avoid secondary infections on the wound for fast wound healing. The wound healing property of chitosan film was improved by addition of other polymers. Thus, the present research work concentrated on the preparation and evaluation of composite films made with chitosan-gelatin at different proportions to ascertain the applicability of prepared combination for wound management as this combination is not yet reported in wound management. .

Chitosan, gelatin combination shown many advantages when used in other preparations like sponges, scaffolds etc. This combination also showed good compatibility in XRD and FTIR studies. Thus the present work is aimed for preparation of ciprofloxacillin loaded composite films to promote wound healing.

Materials and Methods

Ciprofloxacin was obtained as gift sample from Integrated Marketing Co., Hyderabad. Chitosan was extracted from crab shells. Gelatin was procured from Qualigens fine chemicals, Mumbai, India. Ethylene glycol was procured from Central drug House, Mumbai.

Microorganisms

Four bacteria were tested for the evaluation of antimicrobial activity of films. These include two gram positive bacteria (*Bacillus subtilis, Staphylococus aureus*) and two gram negative bacteria (*Eschericia coli, Pseudomonas aeruginosa*) obtained from NCL, Pune. These were maintained in nutrient agar media.

Methods

Extraction of chitosan from crab shells

Crab shells were washed thoroughly to remove sand and other impurities and dried well. These were soaked in 5% sodium hydroxide solution, in 3N hydrochloric acid solution overnight for deproteinization and to remove calcium carbonate.

The shells were treated with acetone for discoloration and dried. The resultant chitin was de acetylated to give chitosan and washed and dried after confirmation of complete deacetylation [19].

Preparation of films

Solvent casting technique

The films were prepared by casting chitosan (1%), gelatin (10%) solution separately and different proportions (1:10, 1:20, 1:30, and 1:40) of both chitosan and gelatin (composite films) as shown in table 1 with 0.2ml. ethylene glycol as plasticizer after vacuum filtration for removal of entrapped air bubbles on the plastic tray and air dried at room temperature[20]. Based on the results of physical parameters of the films, the ratio of 1:40 was selected for preparation of the drug loaded films.

Preparation of drug loaded chitosan-gelatin composite films

Stock solutions of ciprofloxacin hydrochloride were prepared in the concentrations of 1, 2, 3, 4 %w/v. Drug solution was added to 1:40 ratio of chitosan and gelatin polymer solution such that 10, 20, 30, 40 µg. of drug was present in 0.19 sq.cm. area of the film respectively. (this area is equal to the area of standard antibiotic disc) as shown in table 1.

Table 1. Composition, thickness, folding endurance and water absorption capacity of prepared films.

Code of the film	No. of parts			Thickness (µm.) (Mean±S.D.)	Folding endurance (Mean±S.D)	Water absorption capacity (%) (Mean±S.D)
	Chitosan	Gelatin	µg of drug/0.19 sq.cm			
Blank films						
F_1	1	-	-	60±0.017	300	1101.87±9.08
F_2	-	10	-	126±0.02	177.6±6.55	This film was dissolved.
F_3	1	10	-	30±0.01	202±5.50	879.60±14.92
F_4	1	20	-	43±0.005	215±3.60	951.18±7.92
F_5	1	30	-	56±0.011	225±3.51	1266.69±8.86
F_6	1	40	-	61±0.01	256±5.50	1411.03±11.99
Ciprofloxacin hydrochloride loaded films						
CF_7	1	40	10	63±0.015	182.6±6.50	1051.72±10.47
CF_8	1	40	20	66±0.005	217±4.35	1073.37±10.51
CF_9	1	40	30	56±0.011	226.6±4.5	1022.79±11.55
CF_{10}	1	40	40	73±0.011	238±6.55	1134.45±11.13

Characterisation of films

Thickness

The thickness of film influence the time required to absorb the polymer into the body. To determine the uniformity in thickness of film and change in thickness film after drug loading, it was measured for each film using screw gauge at three different sites of the film and the mean was calculated.

Folding endurance

It was determined to find the flexibility of film which is needed to handle the film easily and for comfortable, secured application of film on the wound. It was determined by repeatedly folding one film at same place till it breaks or folded up to 300 times manually. The number of times of film could be folded at the same place without breaking give the value of folding endurance. To find the effect of drug loading on the film's flexibility, drug loaded films were also subjected to folding endurance test.

Water absorption capacity

It is of utmost importance, if they are used for biological applications and wound healing. It is used to measure the capacity of blank and drug loaded films to absorb wound exudates. Preweighed, one inch film was placed in 15ml. of distilled water and the weight of the film was noted periodically at first hour, second hour, third hour and 24^{th} hour. Every time after noting the weight, the film was placed in fresh water. Water absorption capacity of the film was

determined in triplicate and calculated using a formula

$$\%\,\text{Water absorption capacity} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

Tensile strength

Tensile strength measures the ability of film to withstand rupture, mechanical pressures or the force required to break the film. Tensile strength of the blank films and optimized drug loaded film (CF10) was determined by using the *Instron* tensile testing machine at SDDC section in CLRI Chennai, India. It was expressed in MPa units.

Table 2. Protocol and percentage of wound contraction at different periods in different groups of *in vivo* wound healing studies.

Group No.	Purpose	Code of film used for application	Wound contraction (%) (Mean±S.D.)			
			7th day	14th day	21st day	28th day
I	Normal control	-	-	-	-	-
II	Wound control	-	21.31±4.20	59.80±2.95	75.91±2.10	92.46±1.11
III	Treatment with chitosan film	F_1	40.64±4.12	68.29±1.49	84.75±1.06	95.49±0.29
IV	Treatment with composite film	F_6	54.33±3.66	76.72±1.33	90.74±0.69	98.78±0.10
V	Treatment with ciprofloxacin loaded film	CF_{10}	59.49±3.61	83.15±2.05	93.01±0.32	99.68±0.06

Drug load and content uniformity

This test was conducted for drug loaded films to find the percentage of drug was loaded into film and uniform distribution of drug in film. Drug load and uniformity was determined by placing one square inch area of the film in water for 2 hours and the quantity of drug in solution was determined by UV/Visible method after filtration. Films were selected in triplicate from three different areas of drug loaded films to find uniform distribution of drug.

In vitro drug release studies:

In vitro drug release studies were conducted to estimate the rate of release of drug from selected drug loaded film (CF10) and to find the time taken to release the total drug. The diffusion studies were carried out using dialysis membrane. To one end of the dialysis tube, the film was placed. The dialysis tube was placed in the beaker (such that the film was dipped in water of beaker) containing 100ml. distilled water which was placed on a magnetic stirrer. 5ml. of sample was withdrawn for every 10 minutes from the beaker and the concentration of drug in each sample was estimated using UV/Visible spectrophotometer at its respective wavelength. An equal volume of fresh distilled water was replaced after withdrawl of each sample.

Bioevaluation of films

Based on the results of above tests, an optimized composite film (F6) was selected among the prepared composite films for drug loading and for further bioevaluation of films.

Evaluation of antimicrobial activity

The measurement of the antimicrobial activity of individual chitosan and gelatin films, an optimized composite film (F6) and all drug loaded films was done by agar diffusion method using selected gram positive and gram negative organisms. The zone of inhibition was determined by placing a definite size of film into discs made in inoculated solidified

nutrient agar medium in a petriplate which were incubated for 24hrs. at 37±1°C. This was done in triplicate with each film for each organism and an average diameter of zone of inhibition was noted. Based on the results of above characteristics, an optimized blank (F6) and drug loaded film (CF10) were selected for *in vivo* studies.

Table 3. Tensile strength parameters of films and skins.

Tensile strength parameters	F_3	F_4	F_5	F_6	CF_{10}	Normal skin	Treated skin
Maximum load (N)	17.80	43.64	33.29	22.71	27.23	30.540	20.100
Maximum extension (mm)	0.67	1.17	1.12	2.67	1.92	31.360	39.530
Elongation at break (%)	2.22	2.33	2.25	5.33	3.83	156.800	197.650
Tensile strength (mpa)	11.13	39.68	41.62	45.42	32.45	4.212	2.680

Evaluation of wound healing activity by in vivo studies

The wound healing activity was evaluated by excision wound model in an adult albino rats. Pathogen free adult female albino rats weighing 150-200 gms. were selected for the study. The wound healing activity was conducted with the protocol as shown in table 2. The animal work was approved by institutional ethical committee.

Table 4. Antibacterial activity of optimized composite and drug loaded films against different organisms.

Code of film	Diameter of zone of inhibition (in cm.) (Mean±S.D.)			
	Bacillus subtilis	***Staphylococcus aureus***	***Escherichia coli***	***Pseudomonas aeruginosa***
Blank films				
F_1	1.70±0.045	1.66±0.09	1.5±0.072	1.52±0.040
F_2	0	0	0	0
F_6	1.38±0.061	1.37±0.060	1.20±0.050	1.26±0.040
Ciprofloxacin loaded films				
CF_7	2.31±0.035	2.38±0.047	2.28±0.06	2.13±0.045
CF_8	2.58±0.055	2.60±0.055	2.46±0.061	2.46±0.05
CF_9	2.82±0.045	2.93±0.055	2.72±0.045	2.78±0.035
CF_{10}	3.09±0.045	3.11±0.045	3.12±0.055	3.04±0.050

The anaesthetized animal was placed on the operation table in normal position. The dorsal fur of the animals was shaved with an electric clipper and the anticipated area of the wound to be created was outlined on the back of the animals on interscapular region i.e, 5mm. away from ears. Full thickness skin from the demarked area was excised to get a wound area of 2 sq.cm. After achieving haemostasis, the wound was blotted with sterile gauze in control group and the respective film on the wound of animals in treatment groups (Group-III, IV, V). Then the following parameters were determined at specific time intervals.

Percentage of wound contraction

Wound healing is a process by which damaged tissue is restored as closely as possible to its normal state and wound contraction is the process of shrinkage of area of the wound. It mainly depends on the repairing ability of tissue which may be reduced due to infections. It was measured to find the extent of reduction in wound area at different periods of treatment by graphical method. Wound area was calculated on 7^{th}, 14^{th}, 21^{st} and 28^{th} post wounding day by counting number of squares of retraced wound area on graph paper. The degree of wound healing was calculated as % closure of the wound area from the original wound using a formula: % closure = $1-(A_d/A_0)$ X 100; (A_0 – Wound area on day zero, A_d – Wound area on corresponding days).

Histopathological studies

Understanding of microscopic changes is important in assessing efficacy of films in wound healing. The skin tissue samples collected by corneal trephiner at 7^{th}, 14^{th}, and 28^{th} day of post-treatment in different groups for evaluation of the extent of wound healing by studying the histopathological characteristics. Biopsy specimens were preserved in 10% buffered formalin. They were processed by routine paraffin embedding technique i.e, 5-6 microns thick sections were cut and stained with haematoxylin and eosin [21].

Photography

The photographs of wound from different groups were taken at specific intervals for visual comparison.

Tensile strength

The skin tissue samples of normal skin, treated skin were collected at the end of the study i.e., after complete healing of the wound and tensile strength of these samples were measured to compare the skin after treatment with normal skin.

Statistical Analysis

The results are expressed as mean ± S.D. Statistical analysis was performed by analysis of variance (ANOVA) test and statistical significance was set accordingly at P =0.05 level.

Results and Discussion

The thickness of blank and drug loaded films at different compositions of gelatin and drug is shown in table 1. All the films have uniform thickness throughout film. The thickness of all films was between 30-126µm. It was found that increase in the concentration of gelatin, the thickness of composite films was significantly ($p<0.05$) increased. There was no significant difference in thickness of drug loaded films when compared to its respective blank composite film indicated that the drug loading has no influence on the thickness of film.

Folding endurance of composite films was significantly increased with increase in concentration of gelatin. A composite film F_6 shown highest folding endurance among all composite films which was lesser than chitosan film (F1) and greater than gelatin film (F2) as shown in table 1. It indicated that there was no improvement in flexibility of chitosan film by addition of gelatin. There was significant difference ($p<0.05$) in folding endurance between optimized blank composite film F_6 and the drug loaded films may be due to alteration of flexibility of films by drug. CF_{10} shown maximum folding endurance among drug loaded films, which indicated that it may have maximum flexibility among drug loaded films as shown in table 1.

The water absorption capacity of composite films was significantly increased with increase in gelatin concentration, which may be due to hydrophilic and swelling properties of gelatin [14,22] as shown in table 1. A composite film F6 has shown maximum water absorption capacity among all the prepared composite films. The water absorption capacity of drug loaded films was not significantly different with change in concentration of drug. It was found that,

there was significant difference in water absorption capacity of blank composite films and drug loaded films.

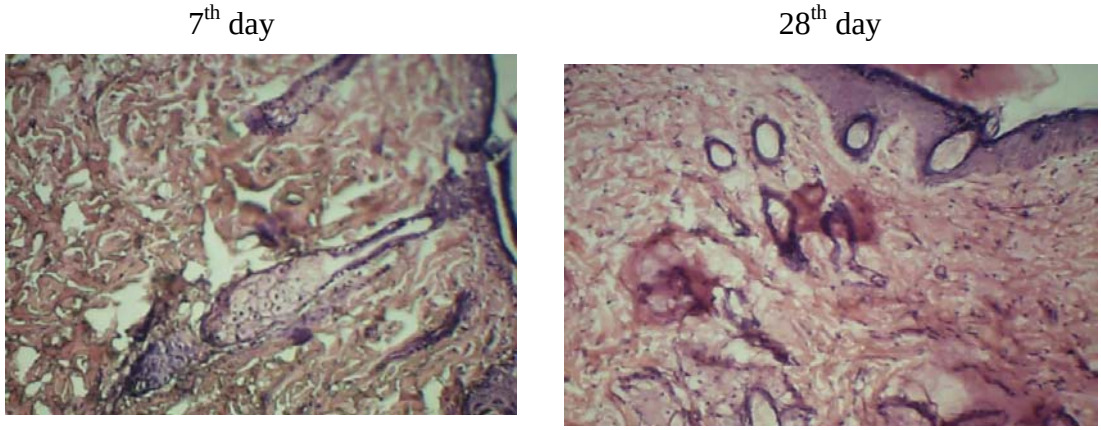

Figure 1. Photomicrographs of wounds treated with chitosan-gelatin composite film.

It indicated that the drug is decreasing the water absorption capacity of film which may be due to interference of drug in water absorption by polymers. Among ciprofloxacin loaded films, CF_{10} shown maximum water absorption capacity. F_6 film has shown maximum tensile strength and maximum elongation which was more than pure chitosan as shown in table 3.

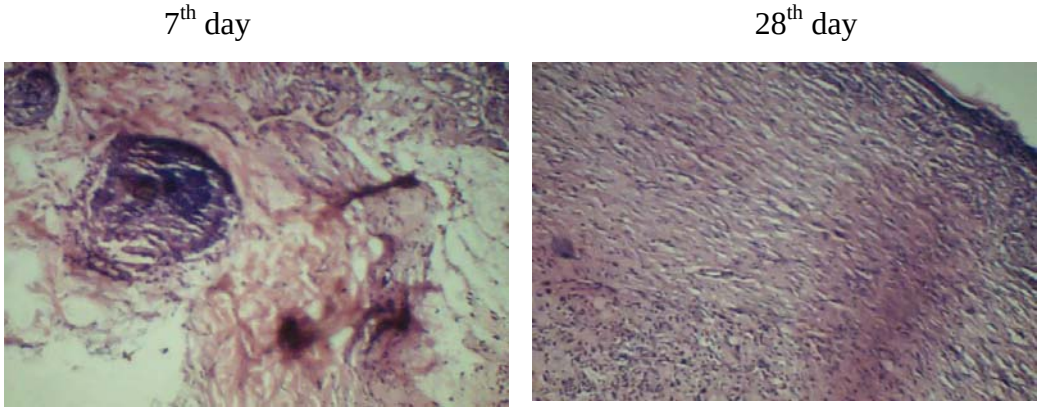

Figure 2. Photomicrographs of wounds treated with ciprofloxacin loaded films.

As gelatin proportion was increased, the values for maximum extension, elongation at break (%) and tensile strength were also increased which indicated that the gelatin improved the strength of film. There was no significant difference ($p<0.05$) in tensile strength of optimized blank and drug loaded films. It indicated that the tensile strength of film was not changed significantly after loading of drug into film.

About mean percentage of 95-99% of ciprofloxacin was incorporated into films. Among ciprofloxacin drug loaded films CF_{10} shown maximum percentage (99.43%) of drug loading. It was found that, there was no significant difference in percentage of drug load into different films though increased concentration of drug/0.19 sq.cms film was used for loading. The percentage of drug content in three sites of drug loaded film was compared to estimate the uniform distribution of drug in the films. There was no significant difference ($p<0.05$). in percentage of drug loaded at three different sites of film, which confirmed the uniform distribution of drug as shown in table 4.

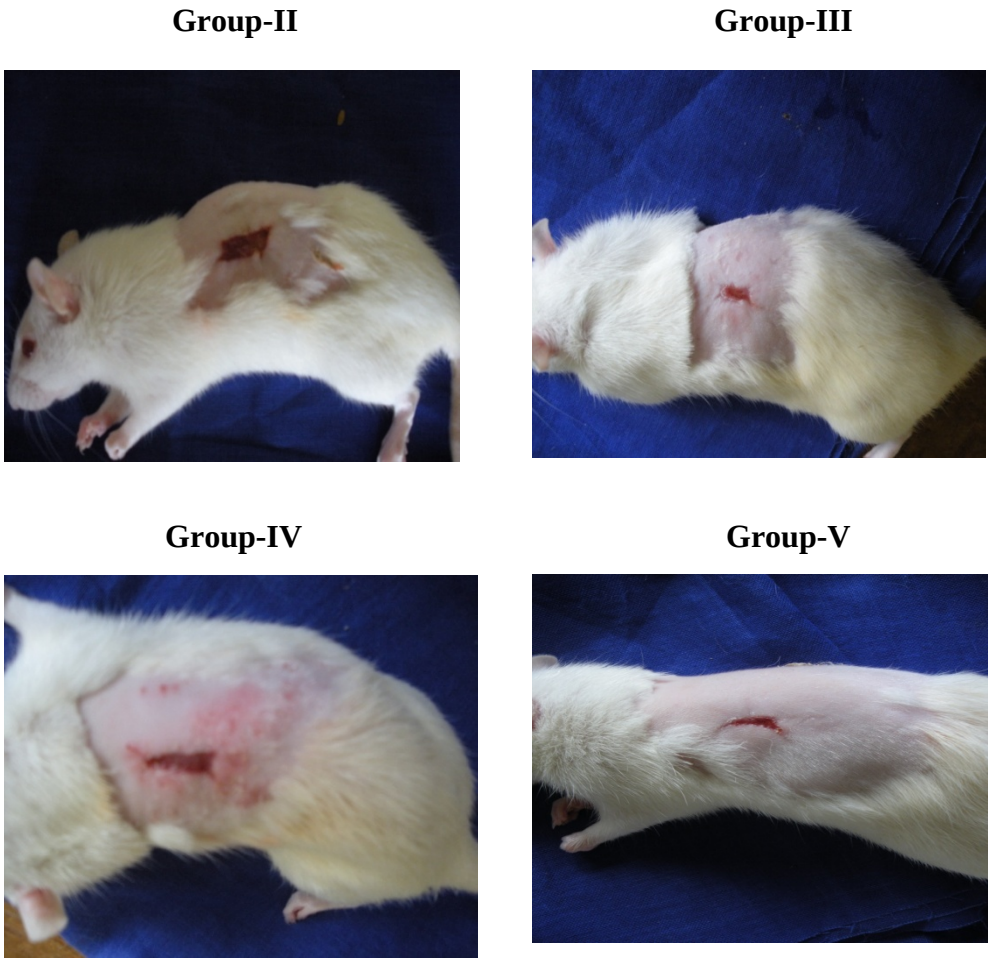

Figure 3. Comparison of photographs of wounds on 14th day in different groups.

Bioevaluation

The zone of inhibition with various films against four different bacterial species was different in agar disc diffusion technique as shown in table 5. There was no inhibition of growth with gelatin film (F2), which may indicate that the gelatin is a protein and cannot inhibit the growth or may promote the growth of bacteria. Zone of inhibition with composite film (F6) was less than with the chitosan film (F1) may be due to gelatin incorporation. As the concentration of drug in the film was increasing the mean diameter of zone of inhibition was also increased with drug loaded films. Among ciprofloxacin loaded films CF_{10} shown maximum inhibition against all tested bacteria. The antibacterial activity of drug loaded films was more than the blank chitosan and composite films. It confirmed that increased antibacterial activity of the drug loaded films than blank films, so drug loaded films can show fast wound healing property than blank films.

Table 5. Drug content and its uniformity in films.

Code of film	Percentage of drug at three different sites of film			
	I	II	III	Mean±S.D.
CF_7	95.00	93.23	97.84	95.36±2.345
CF_8	94.86	96.98	96.10	95.98±1.065
CF_9	97.25	99.02	96.10	98.55±1.471
CF_{10}	98.60	99.91	99.79	99.43±0.72

The percentage of drug release from the selected drug loaded film (CF10) was determined by *in vitro* diffusion studies to find the time taken by film to release the complete loaded drug for eliciting its antibacterial action on wound. 99% of ciprofloxacin was released within 1 ½ hour (90 minutes) indicated that the film is not interfering in drug release on wound as shown in table 6.

Table 6. Cumulative percentage drug release of selected ciprofloxacin loaded film (CF10).

Time (min)	(%) Drug release (Mean±S.D.)
0	0
10	16.36±1.78
20	32.62±1.37
30	36.94±0.79
40	47.04±1.21
50	57.61±1.07
60	68.67±0.81
70	78.84±0.73
80	89.77±0.40
90	98.00±0.21

In vivo studies

The percentage of wound contraction in untreated and treated groups was measured and the results are shown in table 2. There was significant difference in percentage of wound contraction between wounds treated with chitosan (Group-III) and wounds treated with optimized blank composite (Group-IV) and drug loaded films (Group-V). It indicated that chitosan-gelatin composite films and drug loaded composite films have improved wound healing activity than chitosan alone film. Wound contraction was significantly increased in wounds treated with composite film when compared to wounds treated with chitosan film and untreated wounds, which indicated that the composite film has shown more wound healing property than chitosan film. Drug loaded films shown increased wound contraction than blank films indicated that loading of drug into composite films augmented the healing of wound than blank composite films. This may be due to broad antibacterial activity of ciprofloxacin which reduces infections and thus fastens the healing of wound.

Damaged cells in epidermis and around the untreated wound was found whereas restoration and recovery of cells was observed in wounds treated with chitosan film (group III), chitosan-gelatin composite film (group IV) compared to untreated wounds (group II) in 28^{th} day photomicrographs. Further rapid epithelialization was found in photomicrographs of wounds treated with composite films indicated the improvement in wound healing activity compared to wounds treated with chitosan film as shown in fig 1. Almost normal cytoarchitecture of skin was observed in wounds treated with ciprofloxacin loaded films on 28^{th} day when compared to all other groups as given in fig. 2. This suggested that ciprofloxacin drug loaded films may have more capacity for fast recovery and rapid epithelialization of skin than untreated and wounds treated with other films. It may be due to its antibacterial action which prevents further infections on wound supporting for fast epithelialization by stimulation of wound healing environment.

Based on the photographs shown in fig 3, it was found that the wound size was decreased by 7^{th} and 14^{th} day and wound treated with composite film has shown fast wound healing than wounds treated with chitosan film. The size of wound was more decreased in group treated with ciprofloxacin loaded film when compared to other groups on respective days which indicated that loading of drug into composite films augmented the healing of wound than blank composite films. As per table 3, increase in maximum extension of treated skin indicates that improved flexibility of skin after treatment. Effect of stress on elongation of skin has shown positive results for treated skin. The percentage elongation of break was more to treated skin than normal skin, suggested that remodeling of skin was in progress. Maximum load and tensile strength of treated skin was less than normal skin indicated slow progress of the strength of treated skin near to normal skin.

Conclusions

A composite film made with the combination of chitosan and gelatin shown improved % water absorption, tensile strength, wound contraction, histopathological characteristics and visual healing in

invivo studies than with the chitosan film. The antibacterial activity against various bacterial species was not improved by combination of gelatin with chitosan as gelatin cannot inhibit the growth of bacteria. Based on above findings it can be concluded that the composite film of chitosan and gelatin at 1:40 proportion is successful wound dressing for wound management with improved wound healing properties than chitosan alone film. Further the ciprofloxacin loaded films shown improved antibacterial activity and wound healing properties in *in vivo* studies by releasing the drug completely with in one and half hour without significantly changing thickness, folding endurance, water absorption capacity, and tensile strength of blank films.

References

1. Anjaiah A, Haragopal V, Raghavender KBP and Chandra Sekhar EL, Effects of full thickness skin grafts and mesh skin grafts on granulating wounds in dogs: An experimental study. Cheiron. 2001; 30: 92-94.
2. Varshney AC, Amresh K and Harpal S. Effect of various biostimulators on clinical wound healing in bovines. Ind Vet J. 1988; 65 : 436-439.
3. Vogelson CT. Advances in drug delivery systems, Nanotechnol feat art. 2001; 4 : 49-50,52.
4. Shi C, Zhu Y, Ran X, Wang M, Su Y, Cheng T. Therapeutic potential of Chitosan and its derivatives in regenerative medicine. J Surg res. 2009; 133 : 185-192
5. Brown DM, Barton BR, Young VL. Decreased wound contraction with fibrin glue treated skin grafts. Arch Surg. 1992;127, 404-406.
6. Daniela E, Camelia EO, Functionalised Chitosan and its use in Pharmaceutical, Biomedical and Biotechnological Research. Chem Engg Comm. 2008; 195: 1269-1291.
7. Doillon CJ, Deblois C, Cote MF, Fournier N, Bioactive collagen sponge as connective tissue substitute. Mat Sci Engg. 1994; 2, 43-49.
8. Shelma R , Willi P and Sharma CF. Chitin nanofibre reinforced thin chitosan films for Wound healing application. Tren Biomat and Arti org. 2008; 22 : 107-111.
9. In-Yong K, Seo SJ, Moon HS, Yoo MK, In-Young P, Kim BC, Cho CS. Chitosan and its derivatives for tissue engineering applications. Biotechnol Adv. 2008; 26: 1-21 .
10. Emir BD, Raphael MO. Perspectives on: Chitosan Drug Delivery Systems Based on their Geometries. J Bioact Compat poly. 2006; 21: 351-368.
11. Saraswathy G, Pal S, Rose C, Sastry TP, A Novel Bio-inorganic bone implant containing Deglued bone, Chitosan and Gelatin. Bull Mat Sci . 2001; 24 : 415-420
12. Senel S and Clure Mc SJ, Potential Applications of Chitosan in Veterinary Medicine. Advan D Del Rev. 2004; 5: 1467-1480.
13. Ueno H, Yamada H, Tanaka I, Kaba N, Matsuura M, Okumara M, Kadosawa T and Fuginaga T. Accelerating effects of chitosan for healing at early phase of experimental open wounds in dogs. Biomat. 1999; 20: 1407-1414.
14. Su CH, Sun CS, Juan SW, Hu CH, Ke WT and Sheu MT. Fungal mycelia as the source of chitin and polysaccharides and their application as skin substitutes. Biomat. 1997;18: 1169-1174.
15. Mohy Eldin MS, Soliman EA, Hashem AI, Tamer TM. Chitosan Modified Membranes for wound Dressing Applications: Preparations, Characterization and Bioevaluation. Tren Biomat and Arti org.2008; 22: 154-164.
16. Koide SS. Chitin-Chitosan: Properties, Benefits and Risks. Nut Res. 1998; 18: 1091-1101.
17. Akane T, Toshiaki N, Hiroshi M. Acceleration of wound healing by gelatin film dressings with epidermal growth factor. J Vete Med Sci. 2005; 67: 909-913.
18. Fatima AD, Modolo LV, Conegero SA, Porto RR., Wound Healing Agents: The Role of Natural and Non-natural products in Drug Developent. Mini Rev Med Chem. 2008; 8, 879-888,.
19. Burrows F, Loumie C, Abazinge M, Onokpise O, Extraction and evaluation of Chitosan from crab exoskeleton as a seed fungicide and plant growth enhancer. American-Eurasian J. Agric & Environ. Sci. 2007; 2 : 103-111
20. Tanwar YS. Formulation and evaluation of transdermal films of salbutamol sulphate. The Dhaka University journal of Pharm Sci. 2005**;** 4.
21. Carleton HM and Drury RAB. Histological technique for normal and pathological tissue 2nd edition, Oxford University Press, London, 1965; 250.
22. Nagwa F, Hanan E, Mina T. Implantable Biodegradable Sponges: Effect of Inter polymer Complex formation of Chitosan-gelatin on the release Behaviour of Tramadol HCl. D Dev and Ind Pharm. 2007; 33 : 7-17.