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Mucoadhesive wound healing film of Doxycycline Hydrochloride

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Abstract

Wound is an injury usually resticted to physical one with disruption of normal continuity of structure. Water vapour permeable films were formulated using sodium alginate, Hydroxy propyl methyl cellulose 15 cps and Carbopol 934P by solvent casting method. This film forms in-situ hydrdogel by absorbing exudates and adheres to keep wound moist and delivers broad spectrum antibiotic Doxycycline hydrochloride for specified period of time. From the results of preliminary trials, propylene glycol was selected as plasticizer, water and/or isopropyl alcohol as solvent and teflon as casting surface. Films were evaluated for swelling index, % elongation, tensile strength, water vapour transmission rate etc. Optimized films were loaded with broad spectrum antibiotic Doxycycline hydrochloride and were evaluated for in vitro dissolution, wetting ratio, water evaporation, mucoadhesion and microbial penetration study. In vivo and histopathology study of optimized batch D-SC3 was also carried out to confirm its efficacy on 2×2 cm^2 full thickness wound created on albino rats (n=6). Significant difference between test and standard formulation was confirmed by paired two sample t-test where test formulation showed 96% wound contraction in 22 days without scab formation whereas only 80% wound contraction was found in standard formulation.

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Wound healing film, Mucoadhesive, Doxycycline hydrochloride, Sodium alginate, Carbopol 934P.

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Introduction

A wound is an injury usually restricted to physical one with disruption of normal continuity of structure. It occurs when integrity of any tissue is compromised such as skin breaks, muscle tearing, burns etc. Approaches used for wound healing are dry approach and wet approach. Conventional wound care involves application of standard plasters or gauze which can

cover and protect the wound. It can later absorb excess wound exudates to keep the wound dry by forming protective scab. The rigid crust of coagulated blood is body`s own wound cover which protects the wound against external factors. Conventional dry approach soaks up that vital fluid and deprives the wound of the growth factors and enzymes needed for healing. Continual reinjury due to removal of gauze during dressing can slow the process of healing significantly. It has been reported that epithelization is retarded by the dry scab and by keeping moist wound condition, the scab formation is prevented which increases the rate of epithelization [1-3] . The principle of moist healing is now accepted in the pharmaceutical industry, where many products have already been developed for the healing of wounds by moist wound therapy. The objective of moist wound therapy is to create and maintain optimum moist conditions.

The biologicals like enzymes and growth factors can be easily transported in presence of wound exudate. Different types of cells in the wound area communicate through these mediators which promotes healing in a coordinated manner. Wound exudate also provides ideal conditions to cells of immune system to destroy invading pathogens such as bacteria, foreign bodies and necrotic tissues. Thus, the rate of infection is retarded.

Doxycycline hydrochloride is most often used broad spectrum antibiotic and bacteriostatic agent. It has also been reported to have antiprotozoal properties. It is used in the treatment of chlamydia, rickettsia, mycoplasma, and some spirochetes infections, in addition to the standard treatment of gram positive or gram negative bacterial infections. Another added advantage of using Doxycycline hydrochloride is that it inhibits metalloproteinases, tumor necrosis factor-alpha converting enzyme and protease activities in human chronic wound fluid, which suggest that topical one-percent Doxycycline hydrochloride treatment improves healing of chronic, diabetic, foot ulcers, presumably by reducing levels of tumor necrosis factor and matrix metalloproteinases [4,5].

Sodium alginate, Carbopol 934P and Hydroxy propyl methyl cellulose (HPMC 15 cps) have in- situ hydrogel forming property by ion induced, pH induced and temperature induced mechanisms respectively. They have good swelling and film forming property. Carbopol provides good elongation and sodium alginate provides good tensile strength. Alginate is anionic linear polysaccharides obtained from brown algae is composed of mannuronates residues and guluronates in varying proportions [6] and possesses wound healing promoting property by maintaining moist environment. It is biodegradable [7] and can be used for moderate to heavy exudating wound treatment [8].

Thus, present study was aimed to develop and characterize wound healing film of Doxycycline hydrochloride using HPMC 15 cps, sodium alginate and Carbopol 934P which can heal the wound fast.

Materials and Methods

Materials

Doxycycline Hydrochloride was gifted by Zydus Cadila Health Care Ltd., Ahmedabad. Hydroxy Propyl Methyl Cellulose (HPMC 15 cps) and Carbopol 934P were purchased from Central Drug House Ltd., New Delhi and Sodium alginate was purchased from S.D. Fine-Chem Ltd., Baroda. Propylene glycol was provided by Central Drug House Ltd., New Delhi. Isopropyl Alcohol (IPA) was provided by ACS chemicals, Ahmedabad. Sodium dihydrogen phosphate, Sodium bicarbonate, Sodium chloride and Potassium chloride provided by S.D. Fine-Chem Ltd., Baroda. Distilled water was used throughout in the study.

Methods

Preparation of wound healing film

Wound healing film was prepared by solvent casting method where preliminary trials included use of

HPMC 15 cps, Carbopol 934P, Sodium alginate as polymers and propylene glycol or polyethylene glycol as plasticizer. Based on preliminary trials, for preparation of polymer mixture film, sodium alginate and/or HPMC 15 cps were dissolved in distilled water & Carbopol 934P in IPA was added separately followed by slowly mixing with continuous stirring to avoid lump formation. Plasticizer in different amount and Doxycycline hydrochloride (0.25%w/v) were then added and allowed to mix homogenously for 1 h using remi stirrer at 100 rpm and poured on teflon petriplate. The resulting solution was kept in vacuum oven till air entrapped was removed from the solution. The solution was allowed to dry at 30°C for 6 h followed by drying in hot air oven at 45°C for 24 h. This drying time was selected on the basis of preliminary trials where initial 6 h drying at 30°C was required to prevent rapid evaporation of IPA for proper film uniformity. The films were formulated with composition shown in Table 1.

Table 1. Composition of wound healing film using HPMC 15 cps, sodium alginate and Carbopol 934P

Batch no./Ingredients	HPMC 15cps (%w/v)	Sodium alginate (%w/v)	Carbopol 934P (%w/v)	Propylene Glycol (% weight of polymer)	Water and IPA (1:1) (ml)
HC3	1	-	1	30	25
SC3	-	1	1	30	25
HS3	1	1	-	30	25
HC4	1	-	1	40	25
SC4	-	1	1	40	25
HS4	1	1	-	40	25
HSC3	1	0.5	0.5	30	25
CHS3	0.5	0.5	1	30	25
SHC3	0.5	1	0.5	30	25
HSC4	1	0.5	0.5	40	25
CHS4	0.5	0.5	1	40	25
SHC4	0.5	1	0.5	40	25

Preparation of simulated wound fluid

The liquid formulation of simulated wound fluid consists of 0.68 gm of NaCl, 0.22 gm of KCl, 2.5 gm of $NaHCO_3$ and 0.35 gm of NaH_2PO_4 in 100 ml of distilled water. The pH of formulated simulated wound fluid was adjusted to 7.4 [9].

Characterization of wound healing film

Determination of degree of swelling by gravimetric method

The film to be tested was cut into 1 x 1 cm^2 size and weighed accurately. It was immersed in simulated wound fluid at room temperature for 1 h. The swollen gel form of the film pieces were pressed gently with cellulose paper to remove the excess water on the surface of the films and weighed accurately. Degree of swelling was calculated using equation (1).

$$Degree\ of\ swelling = \frac{(W_s - W_d)}{W_d} \times 100 \quad (1)$$

Where, W_s and W_d are weight of film at swollen state and dried state respectively.

Determination of mechanical properties

The folding endurance was measured manually. A strip of film having an area of 2×2 cm^2 was cut evenly and repeatedly folded at the same place till it breaks or cracks. The number of times the film can be folded at the same place without breaking or cracking was considered as folding endurance.

Tensile strength and % elongation of films were evaluated using a tensile testing machine (EIE Instruments, Ahmedabad). Film strip in 2×6 cm^2 of

dimension, free from physical imperfections, was held between two clamps positioned at a distance of 2.5 cm. During measurement, the film was pulled by bottom clamp at a rate of 25 inch/min. The force and elongation were measured when the films broke. Measurements were run three times for each film. The tensile strength and elongation at break were calculated as below using equation (2) and (3).

$$\text{Tensile strength}(Ncm^{-2}) = \frac{\text{Force at break}(Kg)\times 9.8}{\text{Initial cross sectional area of sample}\ (cm^2)} \quad (2)$$

$$\%\ \text{Elongation} = \frac{\text{Increase in length(mm)}}{\text{Original lenth(mm)}} \times 100 \quad (3)$$

Water vapor transmission studies

Water vapor transmission rate (WVTR) was determined according to the ASTM (American society for testing and materials) method E96-90, Procedure D [10]. The apparatus consists of a glass chamber with a cover, isothermal bath at 35°C to prevent condition change, a digital hygrometer to display percentage relative humidity (RH), temperature and dew point, and a reservoir of a saturated magnesium chloride solution. A permeability cup (cylinder, 3 cm in diameter and 5 cm in height) made from high-density polyethylene (HDPE) was placed in the closed glass chamber. 20 ml of distilled water was filled in the permeability cup and the film was fixed onto its brim with adhesive. Evaporation of water through the film was monitored by measuring loss of weight of the cup after 24 hrs [11]. An open cup was used as the control. The relative humidity in the apparatus after equilibration was approximately 40%. Rate of water transmitted was calculated using equation (4).

$$WVTR\ (gm.m^{-2}.d^{-1}) = \frac{W\times 24}{S\times \Delta T} \quad (4)$$

Where, W is Gram of water transmitted in specific time (weight loss), S is exposed surface area (m^2) of the film and ΔT is time duration in hours.

Wetting ratio

To determine wetting ratio, an aliquot of water of 20 µl was dropped on the film. The wetting area (i.e. the water diffusion area) expanded with the time was monitored [12]. The wetting ratio can then be calculated by following equation (5).

$$\text{Wetting ratio} = \frac{\text{Water diffusion area at time t}}{\text{Initial water diffusion area}} \quad (5)$$

Rate of evaporation of water from gel

Hydrogels formed from film were kept at 37°C and 35% relative humidity. After regular intervals, the weight was noted [13] and weight percentage was calculated by the equation (6) (n=3).

$$\text{Weight remaining}\ (\%) = \frac{Wt}{Wo} \times 100 \quad (6)$$

Where, W_o and W_t are initial weight and weight after time't' respectively.

In-vitro dissolution test

The drug release from the film was measured using Modified Dissolution Apparatus. One side of sample film was attached to the teflon plate which was immersed into 500 ml simulated wound fluid at 37±0.5°C as the dissolution medium and stirred at the paddle speed of 50 rpm. 5ml of the sample was withdrawn from the medium at various time intervals. [14-16]. The concentration of drug released was determined by UV spectrophotometer (Shimadzu UV 1800, Japan) at 270 nm [17]. The test was conducted in triplicate and the average result was obtained.

Ex Vivo Evaluation of mucoadhesion strength

Mucoadhesion strength of the films was measured by the QTS Texture Analyzer (Brookfield Engineering Laboratories, Inc., USA). Full thickness rat skin was excised as a model membrane and simulated wound fluid was used as moistening fluid. Excised skin was used immediately for mucoadhesion study. The rat skin mucosa was mounted on the base of Texture analyzer. Tissue holder was used for mucoadhesion

study where rat mucosa was fixed using lid and screw so that no movement of the tissue from the holder occurred during measurements. All measurements were conducted at room temperature of 35°C and relative humidity 50–60%. During measurement, 2 ml of simulated wound solution was evenly spread on the surface of the tissues. Compression test was carried out with 5 gm trigger point and test speed of 30mm/min having 120 second of hold time. The work of adhesion and peak detachment force was used to evaluate the mucoadhesion strength of the film. The Texture expert software recorded the data when the probe started withdrawing from the film. The peak adhesive force and the area under force distance curve (work of adhesion) obtained from the texture profile were used to assess the mucoadhesion of the extruded films. Each measurement was repeated three times using equation (7).

$$Bond\ strength\left(N/cm^2\right)=\frac{Force\ of\ adhesion}{Surface\ area} \quad (7)$$

Microbial penetration study

The ability of films to prevent microbial penetration was tested by placing the films on open 10 ml vials containing 5 ml of nutrient broth and held in place with a screw lid (test area = 0.8 cm²). The negative control was a vial closed with cotton ball while the positive control was an open vial. The tested vials were placed in an open environment for 1 week. The cloudiness of the nutrient broth in any vial was recorded as microbial contamination. [18].

Wound healing test

For in vivo study twelve female Albino rats, weighing about 250-300 gm, were used for the experiment. All the rats were housed singly, to prevent fighting and attack on the wounds. They were adjusted to the setting at least a week to 12 h each of light and dark cycle at room temperature and food and water ad libitum was given before using them for experiments. Caps, sterile gloves and gowns were worn while handling the rats [19]. These studies were approved by Institutional Animal ethics committee (IAEC) (project number IPS/PCEU/MPH10-11/2004). The albino rats were anaesthetized with Diethyl ether. Dorsal hair of the rats was removed with an electric razor. 70% alcohol was employed to sterilize the dorsal area of the animals. Full thickness wound with a surface area of 2×2 cm² was created by removing both dermal and epidermal layer down to the muscle layer from the back using sterilized scissor under aseptic condition [12]. Wound in standard group (n=6) was covered with the commercial product (Povidone-Iodine ointment) for comparison with the test group (n=6) which was covered with optimized wound healing film. Replica of wound size was drawn on butter paper and was replicated on a graph paper to measure wound area for percentage wound contraction study.

Treated rats were placed in individual cages and wound healing was observed on the 1st , 4th , 8th, 12th , 18th and 22nd day using a digital camera. The degree of healing was expressed as the wound contraction ratio (WCR) as shown in equation (8).

$$WCR = \frac{A0 - At}{A0} \times 100 \quad (8)$$

Where, A_o and A_t are respectively the initial area and the wound area at time t.

Wound tissue was dissected, fixed with 10% phosphate-buffered formalin, and stained with Hematoxylin and Eosin (HE) reagents for histological observations.

Results and discussion

Preliminary trials revealed that sodium alginate and/or HPMC 15 cps film can be formulated by distilled water and Carbopol 934P film by IPA using teflon as casting surface. Film formulated using Carbopol 934P and poly ethylene glycol was sticky. Film formed using Dibutyl phthalate were oily and could not be separated as dibutyl phthalate is a poorly hydrophilic plasticizer. Complete film

separation could not be obtained using triethyl citrate. Thus, propylene glycol was selected as best plasticizer for film formulated using Carbopol 934P.

Evaluation of degree of swelling of films

Films formed from individual polymers showed large difference in swelling behavior. Carbopol 934P films showed highest swelling but HPMC 15 cps and sodium alginate showed very less and almost similar degree of swelling. The same result was obtained for composite film as shown in Table 2 where mixture of HPMC 15 cps and sodium alginate showed less degree of swelling whereas swelling was higher when Carbopol 934P was mixed with HPMC 15 cps [20] or sodium alginate. Swelling behavior in same percentage concentration of polymer showed increase in swelling as concentration of plasticizer i.e propylene glycol was increased.

Table 2. Results of various evaluation parameters for selection of batch for drug loading

Batch no./ Evaluation parameters	% Elongation	Tensile Strength (N/cm^2)	Swelling Index	Water Vapour Transmission Rate (gm/m^2day)
HC3	171.2121 ± 32	147 ± 25	11.66667 ± 0.9	1936.306 ± 55
SC3	15.909 ± 3	1850.975 ± 260	11.61559 ± 0.7	2513.8 ± 25
HS3	4.5454 ± 0.9	1092.7 ± 113	1.39 ± 0.11	1970.276 ± 94
HC4	203.03 ± 29	298.9 ± 25	13.875 ± 0.85	2038.217 ± 60
SC4	23.636 ± 3	1773.8 ± 210	13.7561 ± 1.5	2683.652 ± 130
HS4	6.818 ± 1.6	317.275 ± 29	2.8 ± 0.56	2038.217 ± 150
HSC3	20 ± 2.1	1217.16 ± 125	1.4497 ± 0.16	1732.484 ± 125
CHS3	20 ± 4	981.96 ± 36	7.4245 ± 0.62	1732.484 ± 108
SHC3	6.818 ± 1.5	1850.975 ± 190	2.0952 ± 0.18	2411.89 ± 136
HSC4	29.545 ± 8	1594.95 ± 164	2.0434 ± 0.35	1834.395 ± 161
CHS4	23.636 ± 9.7	626.22 ± 50	7.4782 ± 1.11	2174.098 ± 80
SHC4	34.090 ± 13.4	1347.5 ± 94	2.4188 ± 0.69	3125.265 ± 176

Evaluation of mechanical property

Increase in amount of plasticizer from 30% to 40% increased % elongation as shown in Table 2, which may be due to bond formation between polymer and plasticizer and which also may be the reason for increase in tensile strength, i.e. anti plasticizing effect of propylene glycol [21]. This anti plasticizing effect was observed in all the polymers and their composite except in HPMC 15 cps film where increase in plasticizer showed decrease in tensile strength. Sodium alginate is the most brittle material amongst all polymers used and thus showed highest tensile strength whereas Carbopol 934P is most stretchable material and thus showed highest % elongation and least tensile strength. All the films possessed folding endurance greater than 100.

Evaluation of Water vapour transmission rate

Water vapour transmission rate is an important parameter which signifies proper moisture level in wound for faster healing. Reported value of WVTR is 204±12 gm/m^2day for normal skin, 279±26 gm/m^2day for a first degree burn, 5138±202 gm/m^2day for a granulating wound [22] and recommended rate to provide adequate level of moisture for wound healing is 2000–2500 gm/m^2day [23]. WVTR greater than this value results in dehydration of wound and lesser than this value results in accumulation of exudates which results in pain and bacteriological growth.

Figure.1. WVTR of films containing 2% polymer at different plasticizer concentration

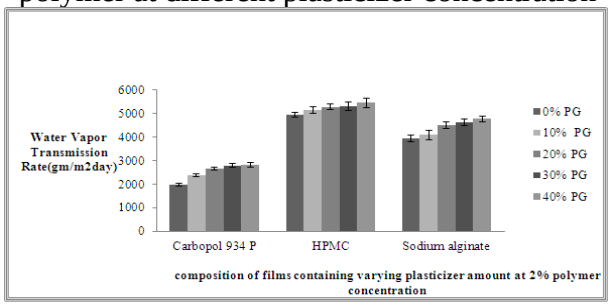


Figure 1 indicates increase in WVTR was observed with increase in plasticizer for all polymers and their composites. Film formed from Carbopol 934P showed less WVTR as compared to Sodium alginate and HPMC 15 cps. This may be due to higher molecular weight and higher viscosity of Carbopol 934P as compared to HPMC 15 cps and sodium alginate. Batches HC4, SC3, SH3, SH4, CHS4 and SHC3 showed WVTR in range of 2000-2500 gm/m^{2}day. Considering all other mechanical properties as observed in Table 2, batches SC3 and HC4 were found to be satisfactory and were used for drug loading. Further evaluation study was carried out for drug loaded batches D-SC3 and D-HC4.

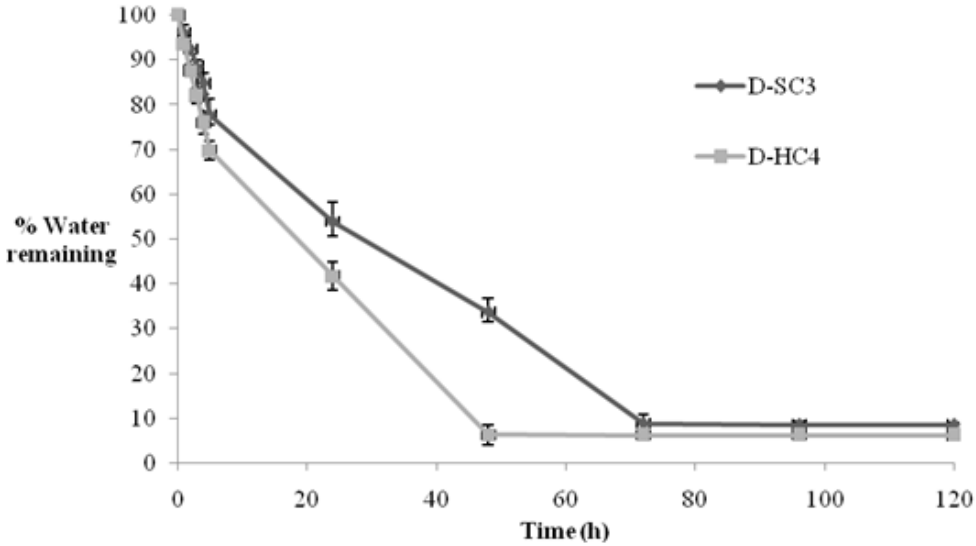
Evaluation of wetting ratio

Wetting ratio is a water diffusion area test which measures wetting area expanded with time which signifies the rate at which wound healing film will absorb exudates during wound healing process. Results showed that when 20 µl of simulated wound fluid was used, it spread to very less extent and formed gel in that region. No significant difference was found between batches D-SC3 and D-HC4 but wetting ratio was found more in D-SC3 batch showing more diffusion which may be due to more water diffusivity in sodium alginate than in HPMC 15 cps.

Evaluation of rate of evaporation from gel

Rate of evaporation from gel is an important parameter particularly when the wound has stopped forming exudates or when the wound has become dry. It is a role of hydrogel that it should not loose significant amount of water within shorter period of time and should not allow the wound to be dry.

Figure.2. Rate of evaporation from hydrogel of batch D-SC3 and HC-4

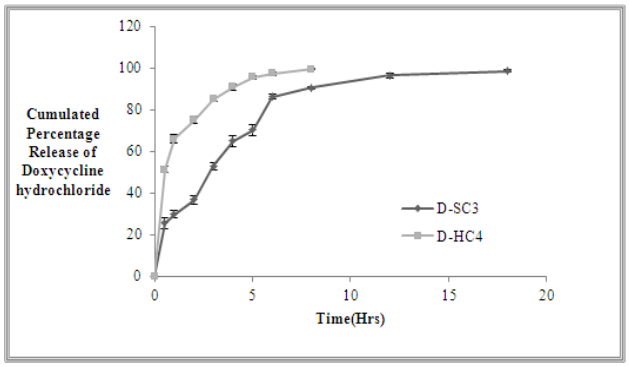


It was observed from Figure2 that the loss of water increased linearly with time for the 1st and 2nd day. After 1st day, the loss was approximately 50% and this increased slowly to about 90% over 3 days. Subsequently, there was no water loss from the gel, and the gel retained about 5-10% of water. Rate of evaporation was found faster in batch D-HC4 than batch D-SC3 which might be due to less water retention property of HPMC 15 cps in comparison to sodium alginate. After 3 days batch D-SC3 was containing 33% of water while batch D-HC4 was containing only 6% water. It can be concluded that batch D-SC3 can keep the wound moist for longer

period and can enhance wound healing better than batch D-HC4.

Evaluation of release profile

Figure.3. In vitro dissolution profile of Doxycycline hydrochloride in batches D-SC3 and D-HC4



In vitro dissolution profile for Doxycycline Hydrochloride as shown in Figure3 indicated batch D-HC4 showed a very rapid release from film and 80% drug was released in 3 h while batch D-SC3 showed 80% drug release in 6 h. Batch D-HC4 showed 50% drug release within first 0.5 h and 90% by 4 h. Batch D-SC3 released 90% drug up to 8 h which is more desirable for wound healing, as it can release drug for longer period of time to prevent infection.

From wetting ratio, rate of evaporation and drug release study it was clear that D-SC3 shows better results than batch D-HC4. So, further evaluations were carried out only for batch D-SC3.

Ex Vivo Evaluation of mucoadhesion strength

Mucoadhesion study was performed to ensure that the film remains on the wound bed irrespective of movement of object. The test was performed on texture analyzer using excised rat skin. Bioadhesion strength and bond strength of batch D-SC3 was found to be 192 gm and 4704 N/m^2, which is enough to prevent film to get detached from the wound bed.

Microbial penetration study

This test signifies the ability of composite film to prevent microbial penetration and prevents secondary infection. In the microbial penetration tests, positive control (P) tube showed microbial growth which ensured that the nutrient broth was suitable for bacterial growth. Negative control (N) tubes showed no growth which ensured the condition that is free from intrinsic bacterial contamination. The test (T) results showed a clear solution, i.e., no visible microbial contamination. This indicates good potential of the batch D-SC3 being used as wound dressing film due to its protective action on the wound from secondary bacterial infection.

Wound healing study of batch D-SC3

Result in Table 3 revealed that batch D-SC3 test film showed appreciable results for parameters like sticking to wound, absorption of exudates and scab formation. Standard povidone iodine ointment did not absorb exudates and could not stick to wound surface. It was also revealed that none of the rats in test group showed scab formation but rats in control group showed formation of scab which delayed wound healing. The test film was clear and transparent thus wound could be observed easily.

Table 3. Results for various in vivo parameters for batch D-SC3 as test film and Povidone- Iodine ointment as standard

Day/ Response	Hemorrhage		Sticking to wound		Scab		Absorption of exudates		Elimination of pus		Edema	
	Film	Std.	Film	Std.	Film	Std.	Film	Std.	Film	Std.	Film	Std.
1	N	N	Y	N	N	N	Y	N	N	N	N	N
2	N	N	Y	N	N	N	Y	N	N	N	N	N
4	N	N	Y	N	N	Y	Y	N	N	N	N	N
8	N	N	Y	N	N	Y	Y	N	N	N	N	N
12	N	N	Y	N	N	Y	Y	N	N	N	N	N
18	N	N	Y	N	N	N	Y	N	N	N	N	N
22	N	N	Y	N	N	N	Y	N	N	N	N	N

Where, Y = Yes and N = No.

Figure.4. Images of wound healing in rats under treatment (Fig.4Ta to Fig.4Tc) using batch D-SC3 as test at various time points of 0, 8 and 22 days and povidone iodine ointment as standard (Fig.4Sa to Fig.4Sc)at various time points of 0, 8 and 22 days.

Fig.4Ta

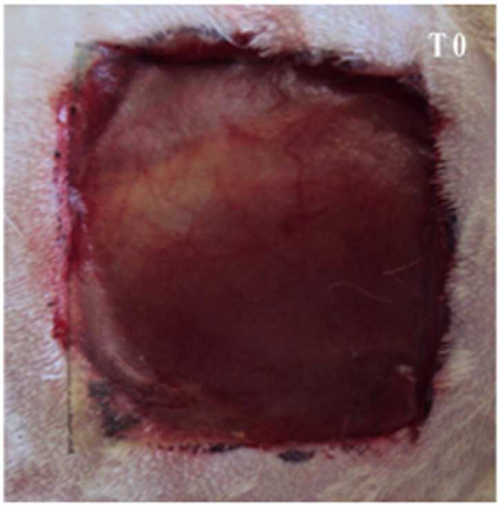


Fig.4Tb

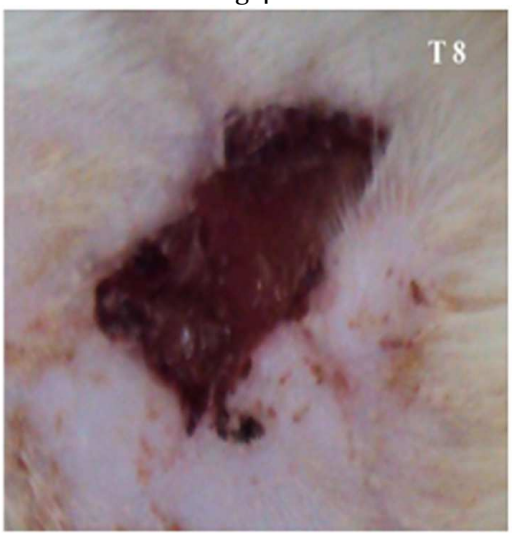


Fig.4Tc

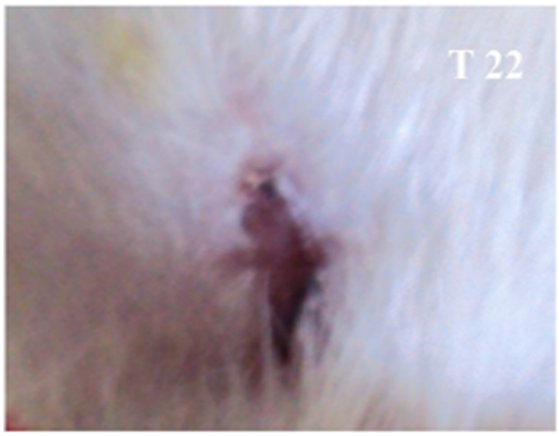


Fig.4Sa

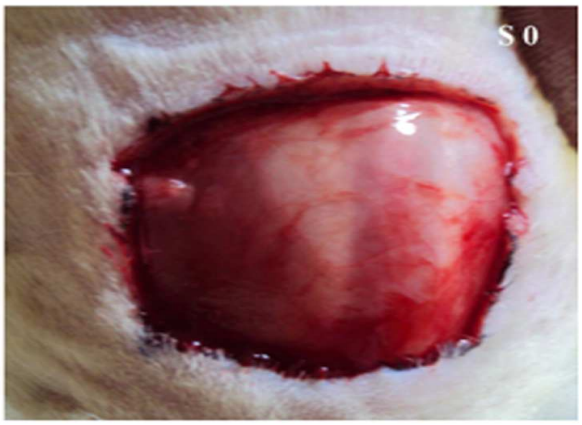


Fig.4Sb

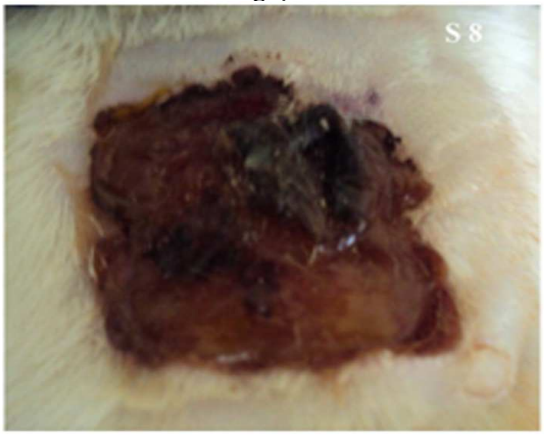


Fig.4Sc

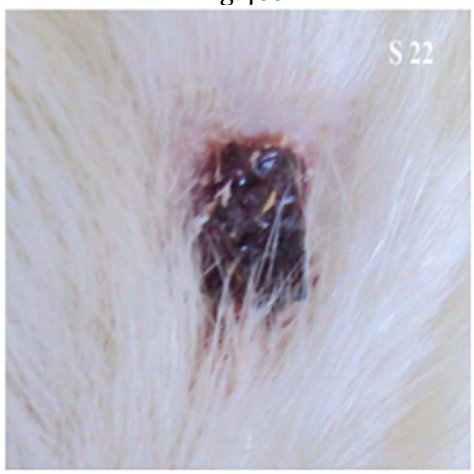
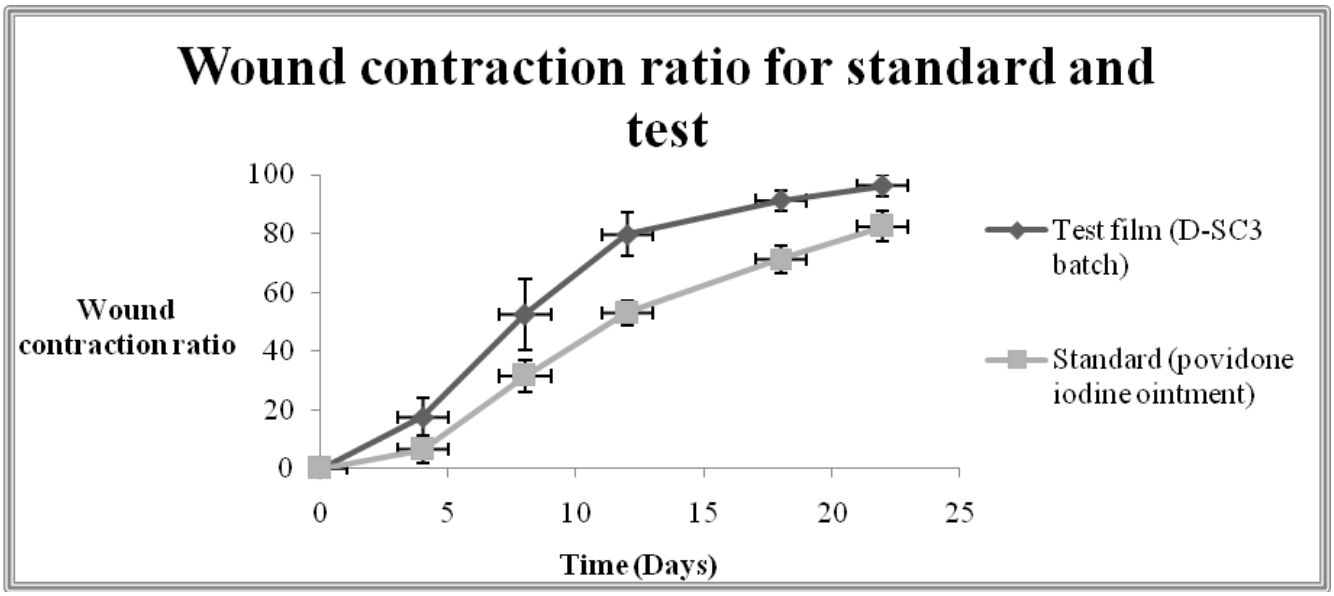


Figure4Ta to 4Tc and Figure 4Sa to Figure 4Sc shows in situ formed hydrogel and redness of test (batch D-SC3) and standard (povidine-iodine ointment) respectively which indicates that vasodilation has occurred in response of chemical mediators and reveals presence of inflammatory response. Scab was clearly visible in standard (Figure4Sb) in comparison to test (Figure4Tb) on 8th day. Higher wound healing in test (Figure 4Tc) was observed on 22nd day in comparison to standard (Figure 4Sc).

Figure 5: Wound contraction ratio for standard and test formulation



Wound contraction ratio shown in Figure 5 indicated 50% of wound healed within 8 days in test group whereas standard group showed 50 % wound contraction after 12 days. At the end of 22 days, 96 % wound was contracted in test group and 82 % wound contracted in standard.

During this period, film was able to adhere at wound surface and maintained moist environment which resulted in fast wound healing without scab formation. During the study period of first 10 days, it was also observed that animals of standard group were having more irritation and were rolling their back to wall of cage while such behavior was absent in test group indicating non irritant effect of the test film. This data was analyzed statistically by paired two sample t-test for means using Microsoft excel 2007. Result revealed that t statistical value (4.0398) was more than t critical value (2.01504), which shows that there is significant difference between standard and test group at 95% confidence interval. So, it can be concluded that test formulation was better than standard formulation as it was able to keep moist environment for fast wound healing.

Histological evaluation

For histology study, excised wound sites were fixed in formalin, processed and embedded in paraffin. Thick sections (3–5 µm) were stained with Hematoxyline and Eosin (H&E). Histological endpoints included a qualitative measurement of the re-epithelialization, inflammatory response and the presence of new vasculature. The presence of new vascular tissue indicated angiogenesis. In the Hematoxylin-Eosin staining procedure, Hematoxylin stains the nucleus, the acidic regions of the cytoplasm and the cartilage matrix to blue. Eosin stains the basic regions of the cytoplasm and the collagen fibers to pink [24]. The photographs were taken by 10X camera microscope using Wincat software ®.

Figure.6. Histological evaluation of wound under treatment using test at various time intervals of 4, 8, 12, 18 days (Fig.6Ta to 6Td) and povidone iodine ointment as standard at various time intervals of 4, 8, 12 and 18 days (Fig.6Sa to Fig.6Sd)

Arrow marks (→) shows Inflammatory response, Star marks

(✧) shows granulation tissue, E shows for Epidermis, B shows Blood vessels, D shows Dermis, C shows Crust.

Fig.6Ta

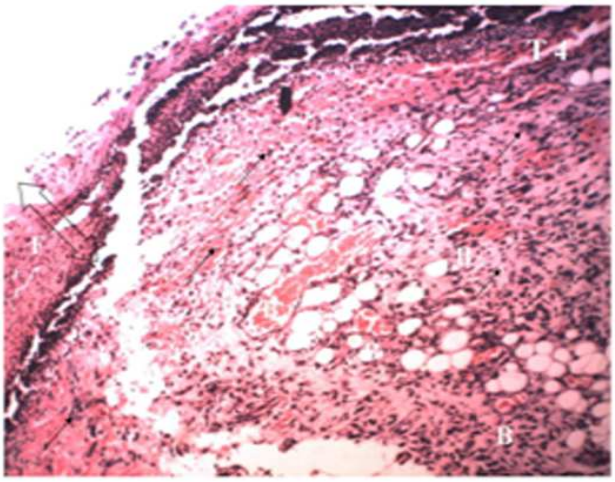


Fig.6Tb

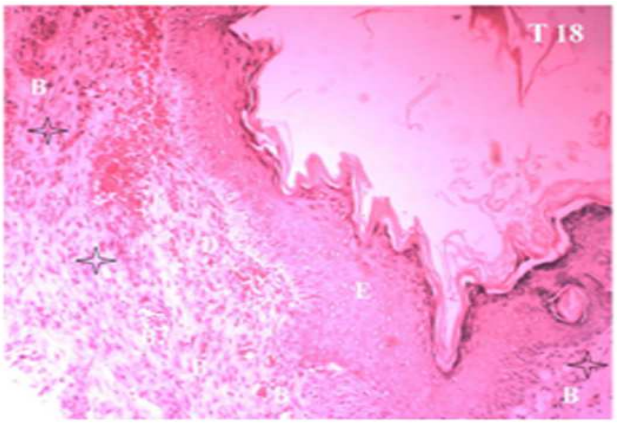


Fig .6Sa

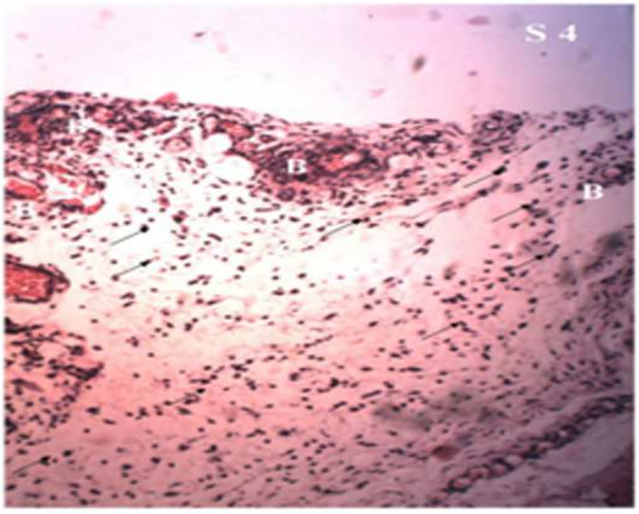


Fig.6Sb

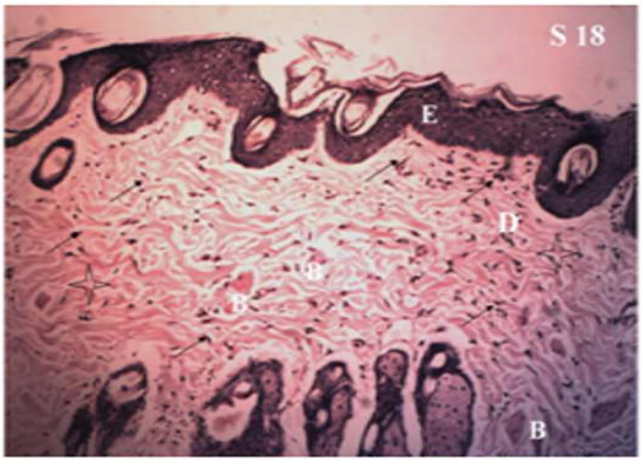


Figure 6Ta to Figure6Tb shows healing pattern of test (batch D-SC3) and Figure 6Sa to 6Sb of standard (povidine-iodine ointment) treated wound after 4 and 18 days respectively. After 4th day, severe inflammation was observed in both test and control wounds. Wound edges were heavily proliferated of the peripheral keratinocytes, which were migrating through the wound bed and infiltrated the bio-integrated film [18]. Dilated blood vessels were observed in both the cases. On the 8th day, film was found to be almost bio integrated in the wound bed while in case of standard, crust was observed. Inflammatory response was present. Some region of section started remodeling of granulation tissues in test while in case of standard, some blank region without any tissues were found which has to be filled by granulation tissues. Angiogenesis was also observed in test wound. On 12th day, new epithelium was observed on the edges with the proliferation of basal layer and new collagen formed in the dermis which appeared mature. Granulation tissue was seen in dermis. The test film treated wound showed the fibroblast rich stage while the control treated wounds still showed continuous infiltration of inflammatory cells.

On 18th day, in test wounds, the defect area became small and filled with fibroblast rich tissue. Inflammatory cells were almost absent. Mature

collagen found present in dermis. However, for control wounds though the surface of the defect was covered with new epithelium, moderate number of inflammatory cells was still present in the upper dermis. Immature collagen fibers filled the dermis. Histologically, it was observed in Figure6Ta to Figure6Td that test film biointegrated during 8 to 10 days in comparison to standard as shown in Figure6Sa to Figure6Sd. Overall result indicated better pattern of wound healing in case of test as compared to standard.

Conclusion

In the present study, wound healing film containing Doxycycline Hydrochloride was formulated using solvent casting method. By maintaining moist environment, faster wound healing was observed using the advantages of in situ hydrogel forming property of polymers like HPMC 15 cps, Carbopol 934P and Sodium alginate by temperature induced, pH induced and ion induced mechanism respectively. WVTR of optimized batch was within required range and rate of evaporation study showed evaporation of unbound water within 3 to 5 days which ensured maintaining moist environment after completion of exudates formation. In vivo study of optimized batch confirmed better wound healing compared to marketed povidone-iodine ointment using paired two sample t-test. Thus, it can be concluded that developed formulation will be highly promising and better alternative for wound healing.

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