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Biodegradable curative film for medical application

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An attempt has been made to produce a herbal based silk fibroin – chitosan biodegradable film. Herbs are used for wound healing purpose, silk fibroin for its remarkable bio-medical and mechanical properties and chitosan for its antimicrobial properties. Herbal extracts, such as *Aloe barbadensis Mill,* (Aloe Vera), *Cissus quadrangularis* (Pirandai), and *Curcuma longa* (Turmeric) have been used to impart wound healing effects. The physical and antimicrobial properties have been studied as per the standard methods. The cytotoxic reactivity of the test, and control sample are evaluated under an inverted phase contrast microscope based on the standard. Scanning electron microscope is used to study the morphology of prepared film for process versatility and highly specific surface area. The chemical compound assessments are studied by Fourier Transform Infrared. Microbial growth under the bio films is found to be absent, and test results show better activity against *Escherichia coli* and *Staphylococcus aureus*. The blended film shows none cytotoxic reactivity to fibroblast cells and test silk fibroin blended film shows slight cytotoxic reactivity to fibroblast cells after 24 h contact. The achievement of numerical more than 2 is considered as cytotoxic effect. Since the film samples achieve a numerical grade not greater than 2, the samples are considered as non cytotoxic. Microbial growth under the bio films is found to be absent and test results show better activity against *Escherichia coli.*

Keywords : Antimicrobial, Biodegradable film, Biopolymer, Chitosan, Silk fibroin, Wound healing

1 Introduction

Biopolymers have generated considerable interest in the field of medical technology since last few decades. These are the materials produced in the nature by herbs, participate in natural bio-cycle and are eventually degraded and reabsorbed in nature. Biopolymers have been projected as versatile candidates in the area of wound dressing, sutures, tissue engineering and drug delivery [1-9]. Silk is a natural fibre group, which is commonly used as biodegradable and biocompatibility materials for wound dressing across the countries. From many years, the silk fibre is used as silk blend for many applications, ranging from textiles to biomedical applications. Medical textile is the one such category of biomedical field. Silk is used in the form of sutures and artificial ligaments in medical applications due to its favourable properties, like natural slow biodegradation, superior mechanical properties, biocompatibility and processability. Many researchers have found silk fibroin to be an ideal platform for various biomedical applications including drug delivery system like in different forms, such as films, hydrogel, fibres & 3D films and wound dressing [10-12].

Chitosan has been found to have an acceleratory effect on wound healing/wound dressing process. Regenerated chitin fibres, nonwoven mats, sponges and films exhibit an increase in wound healing by over 30 per cent. Standard silk coated with chitosan shows wound healing activities [12-19]. The asymmetric chitosan film showed prolonged antibacterial activity and decreased potential silver toxicity. Also, it ensures excellent oxygen permeability, controlled evaporative water loss and enhanced fluid drainage ability also the chitosan film with sustained antimicrobial capability by the dry and wet phase separation method [20-25].

Natural herbs are a reliable source for the treatment of diseases. According to WHO, approximately 80% of the people depends on traditional medicines for their primary healthcare. The drug industries also depend on plants for new drugs because synthetic medicines produce side effects. Hence, researchers show interest in identifying the plants that are used in traditional remedy and evaluating their beneficial activities[26-30]. In the recent times, valuable scientific support has been given in order to authenticate the use of the plants in disease treatment and also to detect the action mechanism of compounds present in the plants. There are several naturally occurring herbal

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plants which aid in healing of wounds. These natural gifts consists of several active materials. These active substances are responsible for healing the wounds[27,28]. *Aloe barbadensis Mill* (Aloe Vera), *Cissus quadrangularis* (Pirandai), *Curcuma longa* (Turmeric), are some of the herbal plants which are easily and readily available in the surroundings and they help in the better healing of wounds. Hence, the different herbs are added as one of the ingredient in the process of the silk fibroin film.

The existing films, used in medical industry, lack the degradation and slow in healing practice, as a result clinically there is a need for the expansion of the efficient and reliable biomaterial film for wound healing with potential for tissue engineering applications. In this work, an attempt has been made to produce the curative biodegradable silk fibroin membrane. Due to the inherent functional properties, silk fibroin and chitosan are used for many medical purposes. The use of traditional herbal medicines along with biopolymers would lead to development of a biodegradable and more effective wound dressing. Hence, the biodegradable herbal embedded natural and biopolymer blended (silk fibroin - chitosan) film based wound dressing has been developed for medical applications. Herbs are used for wound healing purposes, silk fibroin for its remarkable bio-medical and mechanical properties and chitosan for its antimicrobial properties.

2 Materials and Methods

2.1 Materials

Silk cocoons were purchased from Silk Board, Coimbatore, Tamilnadu. Chitosan was purchased from Otto Chemicals, Mumbai, India. Acetic acid, glycerol, polystyrene petri dish plate chemicals were purchased from Sigma Aldrich. The herbs were collected from natural resource around Coimbatore, Tamilnadu, India.

2.2 Preparation of Silk Fibroin Solution

Raw silk cocoons were cut into small pieces and then boiled for 60 min separately. The mixture was treated separately with 300 mL of 0.5% w/w aqueous solution of sodium carbonate at 90-100°C for 60 min. Silk fibres were washed with hot water and dried overnight. The degummed silk was dissolved in the ternary solvent system of calcium chloride ($CaCl_2$) / ethanol (CH_3CH_2OH) / Water (H_2O) (1:2:8 in molar ratio) at 80°c for 6 h. The material must be stirred intermittently to provide better dissolution of silk fibroin. This solution was dialyzed for 3 days in distilled water using a dialysis bag for removing the salts. The distilled water should be changed every day for the better removal of salts. Then the resulting solution was concentrated to get the silk fibroin.

The specifications of hi media LA387-1MT Dialysis membrane 50 have a average flat width of 24.26 mm; average diameter is 14.3 mm and capacity of tube is approximately 1.6 mL/cm.

Dialysis works on the principle of diffusion of solutes and ultra filtration of fluid across a semi-permeable membrane. Diffusion is a property of substances in water; substances in water tend to move from an area of high concentration to an area of low concentration.

2.3 Preparation of Chitosan Solution

Chitosan solution was prepared by dissolving 1g of chitosan in 100 mL of distilled water. To this, 5mL of acetic acid and 7 mL of glycerol were added. The whole solution was prepared by using magnetic stirrer, continuously stirring for 6 h.

2.4 Herbal Extraction

Solvent extraction refers to separating the active substances by physical or chemical means with the aid of a solvent. Ten gram of herb powder was added to 100 mL of methanol in a conical flask and plugged with cotton wool. After 24 h, the supernatant was collected, the solvent was evaporated and the crude extract was stored at 4^oC .

2.5 Preparation of Films

The simple film casting technique is used to prepare the films with 1:1 blends ratio. The samples were prepared with different ratios of silk fibroin, chitosan (with glycerol as a plasticizer), and required concentration of herbal extracts [*Aloe barbadensis Mill,* (Aloe Vera), *Cissus quadrangularis* (Pirandai), *Curcuma longa* (Turmeric)]. The prepared solutions were poured on to 10×2 cm glass plates before drying in an oven at 40° C to obtain the blended films. The films were carefully removed from the plate and stored in the desiccators for future use and testing. The following samples were prepared:

- Chitosan (CS) (100%)
- Chitosan (CS) + silk fibroin (SF) (1:1)
- Chitosan (CS) + silk fibroin (SF) + *Aloe barbadensis Mill*, (Aloe Vera) (1:1)

- Chitosan (CS) + silk fibroin (SF) + *Cissus quadrangularis* (Pirandai) (1:1)
- Chitosan (CS) + silk fibroin (SF) + *Curcuma longa* (Turmeric (1:1)

2.6 Evaluation of Film

Thickness (ASTM D-882)

The thickness of the chitosan, fibroin and herbal blended film was measured using micrometer with least count of 0.001mm prior to all the tests. The film thickness was measured using a micrometer at five locations (center and four corners) by reading accurately, and mean thickness was taken.

Weight of Film (ASTM D-882)

To determine weight uniformity of the each film, five specimens of size 2.0 cm of all films were weighed on electronic balance and mean weight was calculated.

Swelling Ratio

Chitosan, fibroin and herbal blends film were cut in 1×1 cm size and dried in vacuum at room temperature (20-27°C) for a week. After initial weighting they were kept in beaker with 50mL of distilled water at 37°C for 72 h. Then the films were taken out after 72 h carefully, and the excess amount of water was removed from the surface of the films with filter paper. Swelling was calculated using the following equation:

$$\text{Swelling \%} = [(w_S - w_D) / w_D] \times 100$$

where w_S is the weight of swollen films in gram; and w_D, the weight of the dry films in gram.

Folding Endurance

It is 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 is determined by repeatedly folding one film at same place till it breaks or folded up to 300 times manually. The number of times a film could be folded at the same place without breaking, is considered as the value of folding endurance.

Degradation Properties

All the dried samples were weighed (w_o) before the experiment and then washed with deionised water after immersing in HCL solvents for different time periods using the area / volume ratio 0.1cm^{-1}. After each immersion, the sample was carefully removed from the medium and weighed after drying at 40°C till constant final weight (w_F) is achieved. The degradation index (D_i) is calculated based on the mass loss using the following equation:

$$\text{\% Degradation} = [(w_O - w_F) / w_F] \times 100$$

where w_O is the initial weight of the film in gram ; and w_F, the final weight of the film in gram

Porosity

The porosity rates of porous films were determined by dissolving the films in ethanol at room temperature (20-27°C). The cut-out porous film was placed in a scaled test tube with the media (ethanol) in such a way that the sample remains fully immersed without air bubble on the surface. The porosity rate of porous films was calculated using the following equation.

$$P = v_1\text{-}v_3 / v_2\text{-}v_3) \times 100$$

where v_1 is the initial volume of ethanol; v_2, the volume of ethanol after film is immersed; and v_3, the volume of ethanol after film is removed.

SEM and FTIR Studies of Film

The morphology and surface topography of the film was examined by SEM. The chemical composition of the film was examined by FTIR (Fourier Transform Infrared Spectroscopy).

2.7 Bio Evaluation of Film

Anti-microbial test was done using agar diffusion method, (AATCC 147 Qualitative Method). Nutrient broth (13 g for 1000 mL) of 50 mL was prepared and autoclaved. Then *Escherichia coli* was inoculated in the media and incubated for overnight.

One gram of agar powder and 13 g of nutrient broth were prepared and autoclaved. Three petri dish and cotton swabs were also autoclaved. The agar media was poured in the petri dish and left for solidification. The *Escherichia coli* was swabbed over the agar media. The positive and negative media control and then test samples were added and incubated for 24 h.

Contact Dermatitis Test

To determine the allergenic property of the film, contact dermatitis patch test was performed. The sensitivity and specificity of the test were 70-80%. The samples (25 cm × 25 cm) were placed on the

Table 1 — Grade notation attributes of contact dermatitis test

Grade	Attributes
Extreme positive (+++)	Coalescing vesicles bullous reaction
Strong positive (++)	Erythema, papules, Infiltration
Weak positive (+)	Erythema, Infiltration, discrete papules
Irritant (IR)	Discrete, patchy follicular or homogeneous erythema with no Infiltration
Doubtful (?)	Faint macular or homogeneous erythema with no infiltration
Negative(-)	No signs of irritation or Erythema

Table 2 — Physical properties of CS/SF/Herbal blend film

Type of films	Swelling ratio, %	No. of foldings	Degradation %	Thickness of film, mm	Mass per unit area, GSM	Porosity, %
Chitosan (CS) (100%)	37	256	47	0.24	0.20	15
Chitosan (CS) + silk fibroin (SF) (1:1)	40	488	56.8	0.28	0.23	20
Chitosan (CS) + silk fibroin (SF) + *Aloe barbadensis Mill,* (Aloe Vera) (1:1)	49	650	82	0.29	0.28	34
Chitosan (CS) + silk fibroin (SF) + *Cissus quadrangularis* (Pirandai) (1:1)	52	860	86.3	0.34	0.29	31
Chitosan (CS) + Silk Fibroin (SF) + *Curcuma longa* (Turmeric) (1:1)	54	560	76.92	0.29	0.27	29

patch of hairless skin of outer upper arm of human subjects for 24 h and prick test was evaluated after 20 min of application to avoid any adverse effects. The samples if causes itching or rashes, it is said to have an allergenic property. The results were observed and reported as per International Contact Dermatitis Research Group (ICDRG) given in Table 1.

Microbial Penetration Test

To test the ability of the film to prevent microbial penetration, the films were placed on 10 mL open vials. The vials contained 5 mL of nutrient broth and held in place with a screw lid. Along with the sample in one vial, a vial closed with tightly packed cotton ball was used as a negative control and an open vial was used as a positive control. Then all the tested vials were placed in an open environment for one week. The cloudiness of the nutrient broth in any vial was recorded as microbial contamination.

Wound Healing Activity

L-929, an established and well-characterized mammalian cell line that has demonstrated reproducible results, was used to study wound healing activity. Culture medium used is the minimum essential medium supplemented with foetal bovine serum assay method; and rationale is wound scratch assay and ethanol is used in test sample preparation. For sample preparation 50 mg of developed film samples was mixed with 1 mL of ethanol and than 10 mg sample was taken out from this, followed by the addition of 1mL of minimum essential media (MEM) with 10% of FBS to prepare a complete solution for wound healing testing.

To study *in vitro* wound healing assay, L929 cells were grown in 24 well plates at a density of 1 × 105 cells/mL and cultured until ~ 80 % confluency. A small linear scratch was created in the confluent monolayer by gently scraping with sterile cell scrapper. Cells were thoroughly rinsed with 1 × PBS to remove cellular debris and treated with different concentrations of test samples. Cell proliferation was monitored at different time points (0 h, 4 h, 18 h, 24 h, 36 h and 48 h) and images of the migrated cells were taken at different time points using digital camera (Nikon, Tokyo, Japan) connected to the inverted phase contrast microscope (Radical instruments, India). Extent of wound healing was determined by the distance traversed by cells migrating into the denuded area. The wound healing percentage for different concentrations at 48 h was calculated using following equation to analyse the percentage of wound healing activity of the treated samples:

$$\text{Wound healing activity, \%} = \left(\frac{\text{Migrated cell surface area}}{\text{Total surface area}}\right) \times 100$$

3 Results and Discussion

The physical properties of the film, such as thickness, weight, swelling ratio, folding endurance, degradation and porosity, are shown in Table 2.

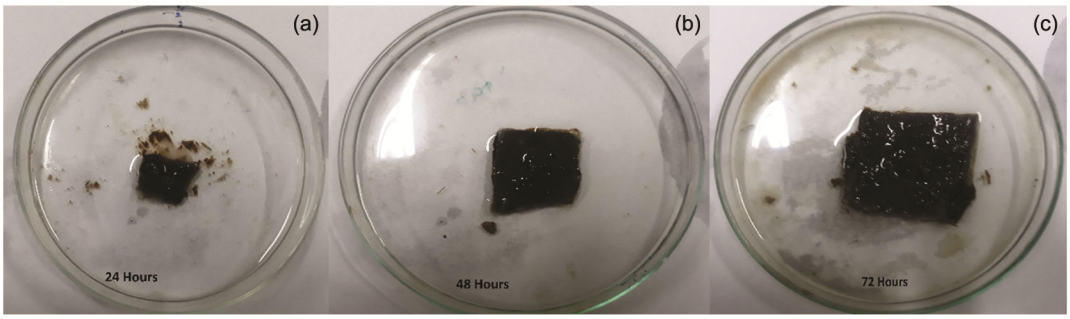

Fig. 1 — Swelling behaviour of chitosan blended with turmeric herbal film (a) after 24 h, (b) after 48 h and (c) after 72 h

3.1 Thickness (ASTM D-882)

The results show that the measured thickness of prepared film, ranges between 0.24 mm and 0.34 mm with higher thickness of 0.34 mm for the combination CS + SF + Pirandai. A notable increase in thickness is observed due to the addition of herbal substance mass, indicating a major role of herb. To obtain the same thickness, same glass plate and an equal volume of the prepared solutions were used, and hence the deviation in thickness was minimized.

3.2 Weight of Film

The mass per unit area of the film was ranged between 0.20 g and 0.29 g. The same trend, as in thickness is observed in mass per unit area. The addition of herbs increases the mass per unit area. Chitosan, silk fibroin and herbal blended film exhibit a mass per unit area range between 0.23g and 0.28 g respectively. Since the method of film preparation is consistent, the weight difference depends on the ratio of herbs, chitosan and silk fibroin present in the film.

3.3 Swelling Ratio

The swelling ratios of chitosan blended films are usually better because of their inherent property. The swelling ratio varies due to different composition percentage of the herbs, and fibroin used in the films. The results show (Fig. 1) that, chitosan blended with turmeric herbal film have 54% of swelling ratio under the standard condition of 72 h, as compared with all other films. On the other hand, the ratio of swelling can be modified based on the composition of chitosan, herbal media and fibroin content; the other chitosan blended films also show better swelling percentage.

3.4 Folding Endurance

Chitosan and Pirandai blended film is found to have the maximum folding endurance of 860, as compared to other films. CS + SF + Turmeric blended film shows the least folding endurance of 560, which means that the films do not break until 560 number of folding. So the film integrity with the skin folding is considered to be very good.

3.5 Degradation

It can be observed that CS + SF + Pirandai blended films show highest degradation percentage than the other films. CS + SF + Turmeric blended film has the least degradation value because the chemical constitution on the herb influences the degradation properties of the film (60–70% carbohydrates, 6–13% water, 6–8% protein, 5–10% fat, 3–7% dietary minerals, 3–7% essential oils, 2–7% dietary fibre, and 1–6% curcuminoids)[11]. Overall, all the bio films have good degradation properties.

3.6 Porosity

The porosity percentage of CS + SF + Aloe Vera blended film is found to be higher when compared to all other films, because the herbal content plays a role to develop the pore gape onto the film surface and core level. Other proportions also give better degradation percentage as compared to chitosan film.

3.7 Surface Characterization

The surface morphology of the film is examined by scanning electron microscopy (SEM). The blended films with different herbs are shown in Fig. 2. The appearance of chitosan, silk fibroin and herbal blended films is found uniform and strong enough to handle without deformation and has better flexibility.

3.8 Chemical Compound Analysis

In order to confirm the structural change, prepared films with different herbs are investigated by FTIR spectra. In the spectrum of chitosan, silk fibroin and *Curcuma longa* (Turmeric) blended film, the characteristics peak at 3323 cm^{-1} is due to the O-H stretch of alcoholic group. Peaks at 2888.38 cm^{-1} and 2049.67 cm^{-1} are due to the O-H stretch in the carboxylic

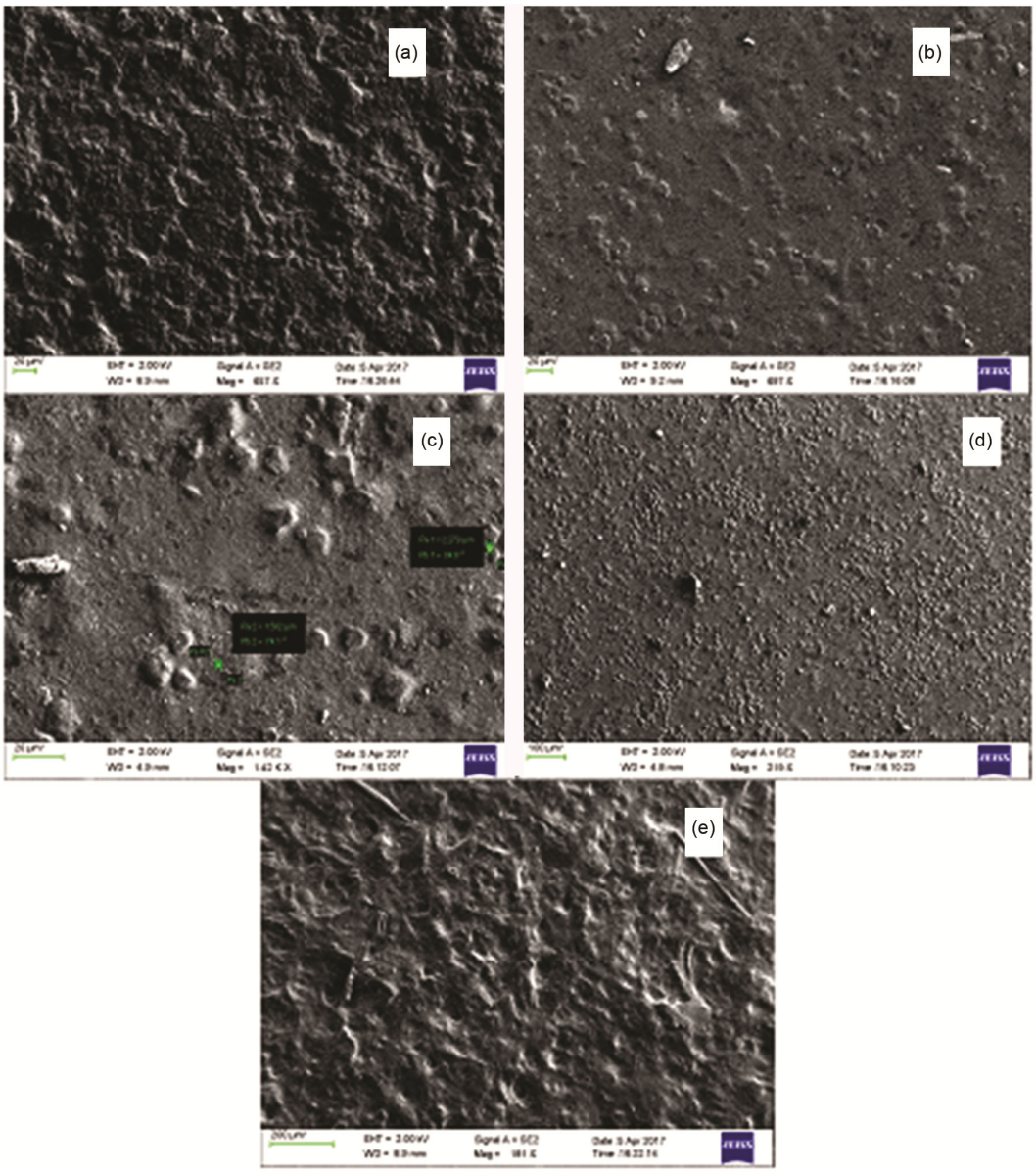

Fig. 2 — SEM image of chitosan and chitosan / silk fibroin / herbal blend films, (a) chitosan, (b) chitosan + silk fibroin, (c) chitosan + silk fibroin + *Aloe barbadensis Mill,* (Aloe Vera), (d) chitosan + silk fibroin + *Cissus quadrangularis* (Pirandai) and (e) chitosan + silk fibroin + *Curcuma longa* (Turmeric) [magnification scale 20µm^{+}]

group. The peaks observed at 1378.21 cm^{-1}, 1319 cm^{-1} and 1151 cm^{-1} indicate the presence of amines. The above peaks confirm the presence of fibroin in traces and the presence of chitosan. Presence of C=O (amide I) stretching is confirmed by the peak at 1632.96 cm^{-1}. The peak 1520.43 cm^{-1} confirms secondary N-H bonding. The peak observed at 1031.10 cm^{-1} corresponds to C-O stretch group and C-C in the carbohydrate structure [18, 19]. In the spectrum of silk fibroin and *Aloe barbadensis Mill* (Aloe Vera) blended film, the characteristics peaks from 849 cm^{-1} to 3260 cm^{-1} are due to bending and stretch. Peak at 3260.42 cm^{-1} is assigned to O-H stretch of the alcoholic functional group; peak at 2938.66 cm^{-1} confirms the O-H stretch of carboxylic functional group; peaks at 1644.10 cm^{-1} and 1552.29 cm^{-1} correspond to the C=O (amide I) stretching and secondary N-H bonding (amide II) respectively. C-N stretch of the amine is observed by the peaks at 1327.09 cm^{-1} and 1103.25 cm^{-1}. The peak observed at 1034.63 cm^{-1} corresponds to C-O stretch and C-C stretch in the carbohydrate structure and the peak at 849.92 cm^{-1} also confirms the C-H bending of

the carbohydrates. C=C stretch of the mono-substituted alkenes is confirmed by the peaks at 991.90 cm^{-1} and 921.68 cm^{-1}. In the spectrum of chitosan, silk fibroin and pirandai blended film, the characteristics peaks from 849 cm^{-1} to 3273 cm^{-1} are due to bending and stretch. The peak observed at 3273.10 cm^{-1} confirms the presence of O-H stretch of the alcoholic group and the peak observed at 2935 cm^{-1} confirms the presence of O-H stretch of carboxylic group. C-N stretch of the amine is confirmed by the peaks at 1334.58 cm^{-1} and 1153.38 cm^{-1}. The above peaks indicate the presence of fibroin and chitosan in traces in the prepared blended films. Peaks at 1632.31 cm^{-1}, 1543.89 cm^{-1} and 1408.07 cm^{-1} correspond to C=O stretching (amide I) and secondary N-H bonding (amide II). Peaks observed at 1237.79 cm^{-1} and 1031 cm^{-1} correspond to C-O stretch and C-C stretch in the carbohydrate structure. Peak at 849.78 cm^{-1} indicates the C-H bending of carbohydrates and the peak at 922.34 cm^{-1} indicates the presence of C=C stretch of mono-substituted alkenes.

Table 3 — Anti microbial test (agar diffusion method)

Type of sample	Zone of incubation, mm	
	S. aureus	*E. coli*
CS (100%)	34	25
CS + SF (1:1)	33	24
CS + SF + Aloe Vera (1:1)	37	26
CS+ SF + Pirandai (1:1)	33	25
CS + SF + Turmeric (1:1)	34	24

3.9 Bio Evaluation of Films

Anti Microbial Test

All the samples have been tested against *Staphylococcus aureus and Escherichia coli,* and the anti microbial test results of the films are listed in Table 3. All the samples were showed excellent (zone of inhibition) antimicrobial property against Gram positive and Gram negative bacteria. The results reveal that, all the samples have a better antimicrobial activity against the Gram positive (*Staphylococcus aureus*) and Gram negative (*Escherichia coli*) bacteria. The film of chitosan, silk fibroin and *Aloe barbadensis Mill,* combination shows high zone of inhibition as compared to all the other films.

Contact Dermatitis Test

Inferences of contact dermatitis test are tabulated in Table 4 as per the test procedure. No allergic reaction is indicated by negative (-), mild reaction is indicated by doubtful (?) and moderate allergic reaction is indicated by (IR). The results explain that one subject experiences allergic irritant reaction to three films, and the conclusion may be arrived that the subject's skin is too sensitive, whereas the films, which used citric acid as solvent show allergic irritant reaction and doubtful in case of three subjects. This might be because of the residual traces of acetic acid in the film even after thorough washing. The irritant reaction gets subsided and become doubtful in the addition of

Table 4 — Contact dermatitis test for films

Subjects	Film Samples				
	CS (100%)	CS + SF (1:1)	CS + SF + Aloe Vera (1:1)	CS+ SF + Pirandai (1:1)	CS + SF + Turmeric (1:1)
Subject 01, (male 19 years)	-	-	-	-	-
Subject 02, (male 26 years)	-	-	-	?	?
Subject 03, (male 23 years)	-	-	-	-	?
Subject 04, (male 25 years)	-	-	-	-	-
Subject 05, (male 22 years)	-	-	-	-	-
Subject 06, (male 19 years)	-	-	?	-	-
Subject 07, (male 21 years)	?	IR	IR	?	IR
Subject 08, (male 20 years)	-	-	-	-	-
Subject 09, (male 23 years)	-	-	-	-	-
Subject 10, (male 22 years)	?	-	?	?	-

Table 5 — Microbial penetration test

Type of film	Microbial contamination	
	Positive control	Negative control
CS (100%)	Yes	No
CS + SF (1:1)	Yes	No
CS + SF + Aloe Vera (1:1)	Yes	No
CS+ SF + Pirandai (1:1)	Yes	No
CS + SF + Turmeric (1:1)	Yes	No

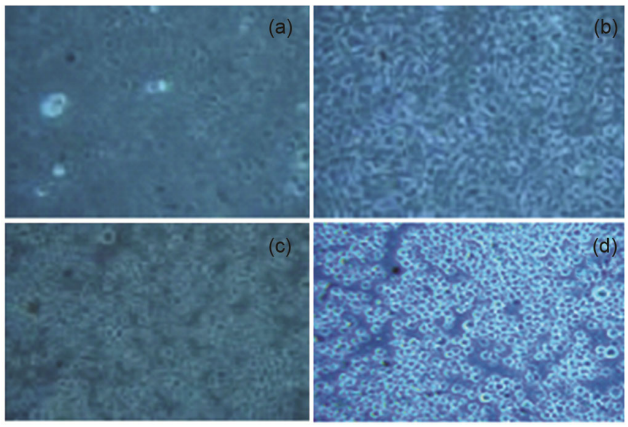

Fig. 3 — Cytotoxicity test before and after staining (a) control sample, (b) CS + SF +Aloe Vera, (c) CS + SF + Pirandai and (d) CS + SF + Turmeric

herbal. However, the allergic reaction is ceased when the concentration of herbal is increased. It can be interpreted that citric acid may cause skin allergies to sensitive people [23, 24].

Microbial Penetration Test

Table 5 shows the results of microbial penetration test of all the developed films. Microbial contamination is not observed in all the formulations of chitosan, chitosan + silk fibroin, chitosan + silk fibroin + *Aloe barbadensis Mill,* chitosan + silk fibroin + *Cissus quadrangularis* and chitosan + silk fibroin + *Curcuma longa* blended films covered tubes and the negative control tubes. Only the positive control tubes show bacterial contamination. This indicates that the developed composite films have good potential for use in wound dressings because of their ability to bind the negatively charged bacteria to the positively charged amino groups of the chitosan polymer and herbs by reducing the primary wound contamination. Hence, the protection of a wound from secondary bacterial infection can be achieved [25,29,30].

In vitro Cytotoxicity Analysis

As per ISO 10993:5, the attainment of numerical grade more than 2 is considered as 30% cytotoxicity effect. The cytotoxic reactivity of the test and control sample are evaluated under an inverted phase contrast microscope based on the standard. Figure 3 shows the cytotoxicity test before and after staining of developed films. The chitosan, silk fibroin and herbal extracts blended films show none cytotoxic reactivity to fibroblast cells and test. Chitosan and silk fibroin blended film shows slight cytotoxic reactivity to fibroblast cells after 24 h contact because of presence of the amino acid in the silk fibroin. The attainment of numerical more than 2 is considered as cytotoxic effect. Since all film samples show a numerical grade not greater than 2, the samples are considered as non cytotoxic. Control gives none cytotoxic reactivity as expected [22–27].

4 Conclusion

The film formation has been done successful by using bio materials, such as chitosan, silk fibroin, and herbal extracts like *Aloe barbadensis Mill* (Aloe Vera), *Curcuma longa* (Turmeric) and *Cissus quadrangularis* (Pirandai) using simple film casting technique. SEM and FTIR results show that structural and chemical characteristics of silk fibroin are not affected while blending with chitosan and herbal extracts, like *Aloe barbadensis Mill*, *Cissus quadrangularis* and *Curcuma longa*. The physical and anti microbial tests are carried out as per the standard methods. The cytotoxic reactivity of the test results show that, the blended film has no cytotoxic reactivity to fibroblast cells but the silk fibroin blended film shows slight cytotoxic reactivity to fibroblast cells after 24 h contact. The achievement of numerical more than 2 is considered as cytotoxic effect. Since the film samples show a numerical grade not greater than 2, the samples are considered as not cytotoxic. Control gives none cytotoxic reactivity as expected. Microbial growth under the bio films is found to be absent and test results show better activity against *Escherichia coli*. This study should be extended in the context of various types of wounds and also the mechanism behind the antibacterial effect may be addressed for the broader perspective.

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